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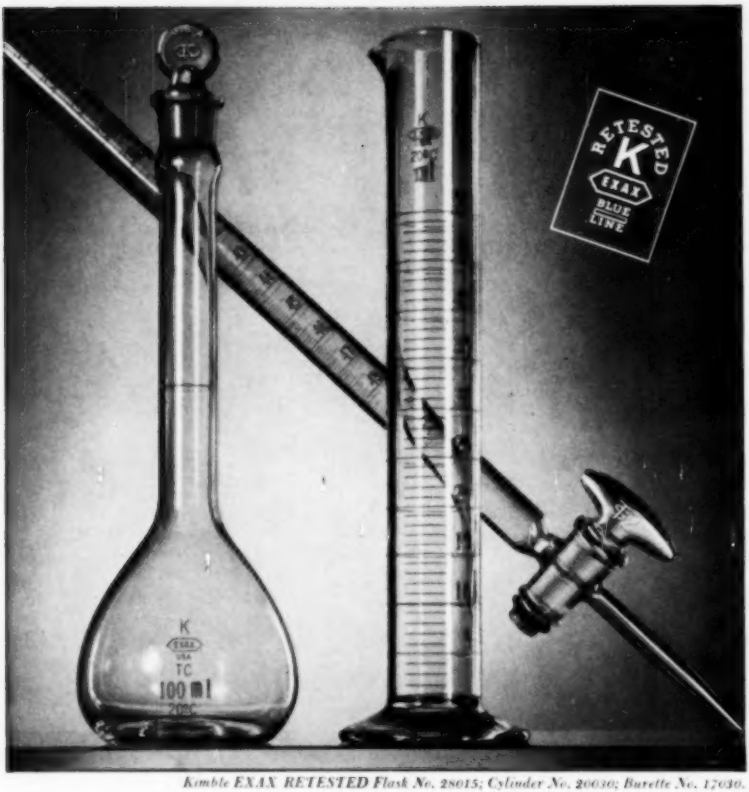

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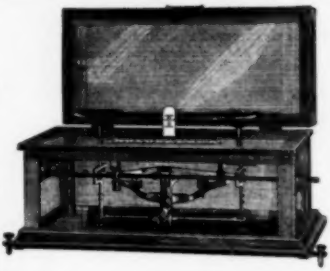

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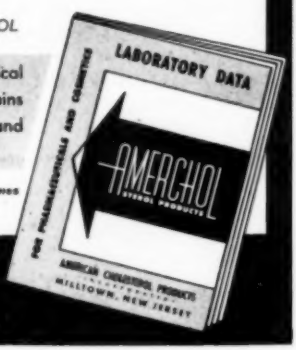

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VOLUME XXXVIII — *MAY, 1949* — NUMBER 5 — CONSECUTIVE NO. 10

Muscle Irritation Following the Injection of Various Penicillin Preparations in Rabbits*

By A. A. NELSON, C. W. PRICE and HENRY WELCH

SINCE the introduction of penicillin as a chemotherapeutic agent numerous preparations have been devised which were designed to delay its absorption from the tissues and thus prolong the effective concentration of penicillin in the blood. Penicillin in oil and wax was one of the first preparations (1) which successfully accomplished this purpose. This product consists of either calcium penicillin or crystalline sodium or potassium penicillin incorporated in a menstruum of peanut or sesame oil and wax. This admixture of penicillin with oil and wax resulted in a delayed absorption of the highly soluble penicillin salt and prolonged penicillin concentrations for twenty-four hours in most individuals injected intramuscularly with 300,000 units. Recently, prolongation of penicillin blood concentrations has been accomplished by using procaine penicillin (2, 3). Procaine penicillin is a relatively insoluble salt of penicillin, and, at present, is used in three dosage forms: procaine penicillin for aqueous injection, procaine penicillin in oil, and procaine penicillin in oil with aluminum monostearate (4). It is possible with the latter product to obtain blood concentration of penicillin for about four days following the injection of 300,000 units. The usual method for administration of these preparations is by the intramuscular route.

During the time that penicillin in oil and wax was widely used numerous complaints were voiced concerning the pain following intramuscular injection, the occasional formation of sterile abscesses, and the persistence of lumps due to the slow absorption of the beeswax. The intramuscular injection of procaine penicillin preparations is relatively painless, due probably to the analgesic effect of the procaine. This lack of pain would not, however, lessen any possible muscle damage caused by the procaine penicillin or other constituents of the product. Accordingly, it was thought desirable to compare the pathological effects of various penicillin products on rabbit muscle. This study includes the preparations already mentioned, the vehicles used in compounding them, and, in addition, other penicillin products less widely used for the prolongation of penicillin blood concentrations. The

* Received Dec. 31, 1948, from Federal Security Agency, Food and Drug Administration, Washington, D. C.

formulas of the products used in this study are given in the following table:

1. Procaine penicillin in sesame oil with 2 per cent *w/v* aluminum monostearate.
2. Procaine penicillin in sesame oil.
3. Procaine penicillin, aqueous suspension, with about 0.5 per cent carboxymethyl cellulose.
4. Vehicles used with procaine penicillin, 2 per cent sodium alginate, 2 per cent *w/v* aluminum monostearate in sesame oil.
5. Penicillin in oil and wax.
6. Aluminum penicillin in oil.
7. Penicillin in oil with epinephrine.
8. Procaine penicillin in oil with pectin and 2 per cent *w/v* aluminum monostearate.

One milliliter of the preparation under test was injected into the sacrospinalis muscle of the rabbit. All of the penicillin products contained 200,000 u./ml. By using the anterior and posterior portions of each muscle it was possible to test four samples per animal. The animals were sacrificed for the most part at seven or at fourteen days, the muscles removed and fixed in 10 per cent formalin.

PATHOLOGY

There were submitted for pathological examination 58 blocks of rabbit muscle fixed in formalin, each block containing an injection site. After gross examination of each muscle block, paraffin sections were made and stained with hematoxylin-eosin. The pathologist did not know the identity of the materials injected.

On both gross and microscopic examination distinct differences were observed between the effects produced by certain categories of the injected compounds, while the lesions caused by others were quite similar in appearance. Unless otherwise stated, the appearances associated with any one compound were not greatly dissimilar in the seven-day as compared with the fourteen-day specimens, and one description suffices for both.

Procaine penicillin in sesame oil with 2 per cent *w/v* aluminum monostearate caused areas of muscle damage averaging about 2.5 x 0.5 x 0.5 cm. The color of the damaged areas, after formalin fixation, was light yellowish brown. Small to moderate numbers of cystic spaces 1 mm. or less in diameter contained a fluid or semifluid colorless oily material. Half the specimens in this group also contained small amounts of creamy whitish material. In a third of the specimens much of the area of injection was of a friable and semifluid character. Microscopically, the injection sites showed moderate amounts of muscle necrosis, small cystic areas containing more or less of amorphous or necrotic debris, inflammatory cellular exudate, fibrosis, and foreign body giant cells. Hemorrhage and calcification were present in only small amounts.

Procaine penicillin in sesame oil caused changes very similar to those described in the previous paragraph, but the degree of damage was possibly just a little less.

Aluminum penicillin in oil, penicillin in oil with epinephrine, and procaine penicillin in oil with pectin and 2 per cent *w/v* aluminum monostearate, all caused changes about as described above; however, material submitted for examination was limited in amount in comparison with the other categories of injected material discussed in this paper.

Penicillin in oil and wax caused lesions whose size was the same as with the compounds described above, but which differed moderately on both gross and microscopic examination. The consistency was firmer, and there was a slight grittiness to touch. Very few small cystic spaces and no creamy material were noted. Microscopically, there was more fibrosis and more walling-off of necrotic muscle than resulted with the compounds described above, and somewhat more numerous foreign body giant cells, while there was less of acute inflammatory cellular exudate.

Very little muscle damage was noted in the fourteen-day specimens following administration of procaine penicillin, aqueous suspension with about 0.5 per cent carboxymethyl cellulose. The lesions at seven days were intermediate in size between the fourteen-day ones and those described in previous paragraphs. No creamy material was noted grossly. Qualitatively, the microscopic appearance did not differ much from that in the penicillin in oil categories, but quantitatively it correlated with its gross appearance in being definitely decreased in amount.

The two vehicles injected separately, namely 2 per cent sodium alginate and 2 per cent aluminum monostearate in sesame oil, showed grossly only small amounts of reddish streaking in the muscles. Microscopically, there was no calcification or foreign body giant cells; other changes as previously noted were present in very small amount. A basophilic jelly-like material was present between bundles of muscle fibers in the alginate specimens. The total amount of damage from the vehicles alone was very small.

COMMENTS

On pathological examination, none of the penicillin preparations included in this study caused more than moderate damage to rabbit sacrospinalis muscle when injected in 1-cc. doses.

All the penicillin in oil preparations, with or without procaine and/or aluminum monostearate, caused gross and microscopic appearances of a quite similar nature.

Penicillin in oil and wax showed damage similar in extent to that caused by the substances just mentioned, but with certain gross and microscopic differences.

Aqueous procaine penicillin caused damage intermediate in amount between that in the above categories and that from the vehicles alone. Damage from the latter was very slight.

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A Study of the Reported Condensation of Chloral with *p*-Chlorobenzenesulfonic Acid. An Improved Synthesis of *p*-Chlorobenzenesulfonic Acid*

By WALTER A. COOK and KATHRYN H. COOK†

The synthesis of *p*-chlorobenzenesulfonic acid has been studied and a preparative method for this compound is described in detail. Three separate methods for the condensation of chloral with *p*-chlorobenzenesulfonic acid are described and evaluated.

A STUDY of the literature on the synthesis of DDT indicated that while many condensing agents have been considered, only sulfuric acid (11), chlorosulfonic acid (4, 13), and hydrofluoric acid (15) have been successfully employed in its preparation. The technical production of DDT has been limited to the exclusive use of the first-mentioned condensing agent, even though it was pointed out recently that the economic operating limit of this method as expressed in terms of the percentage theoretical yield of pure DDT, is appreciably lower than that by the chlorosulfonic acid method (5). In 1945 an article by Giral and Rangel (7), in an obscure periodical, announced the synthesis of DDT in quantitative yield from chloral and *p*-chlorobenzenesulfonic acid according to the following reaction: $CCl_3CH(O)+2p\text{-}ClC_6H_4SO_3H \rightarrow H_2S_2O_7+CCl_3CH(p\text{-}C_6H_4Cl)_2$.

Moreover, these authors postulated that the condensation of chloral with chlorobenzene in the presence of sulfuric acid occurs through the formation of an intermediate compound, *p*-chlorobenzenesulfonic acid and therefore they believed the quality of the DDT technical product might be improved through the direct synthesis of the insecticide according to the above-indicated equation.

The singular nature of this claim together with the long-recognized stability of the C—S bond in aryl sulfonic acids, suggested to the authors that this work be re-examined to determine whether it could be substantiated.

EXPERIMENTAL

Since the literature references on *p*-chlorobenzenesulfonic acid (1, 2, 6, 10, 12) disclosed no information as to yields, and no actual isolation or purification of the free acid from either the sulfonation reaction product or from any of the several salts prepared and described, the development of a satisfactory synthetic procedure for *p*-chlorobenzenesulfonic acid became necessary as a preliminary to the intended condensation studies of this compound with chloral. Accordingly, the details of the preparation of *p*-chlorobenzenesulfonic acid are given as follows:

In a three-necked flask fitted with a mercury seal stirrer, dropping funnel, thermometer, and gas exit tube, was placed 225 Gm. chlorobenzene (2 moles). The flask and contents were cooled to 0° in an ice-salt water bath and 230 Gm. chloro-

* Received Oct. 26, 1948, from the Knight Chemical Laboratory, University of Akron, Akron, Ohio.
† The authors acknowledge the courtesy of Mr. Hector H. McAllister for translation service, and Mr. F. von Bergen of the Westvaco Chlorine Products Corporation for the sample of technical chloral, used in this investigation.

sulfonic acid (2 moles) added dropwise over a sixty-minute interval with the temperature of the reaction mixture being maintained at 10–15° for a total stirring period of six hours. The contents of the flask were then poured into 500 ml. saturated NaCl which in turn was surrounded by an ice salt bath. The precipitated sodium *p*-chlorobenzene sulfonate was filtered on a Büchner funnel, and air-dried to a constant weight of 377 Gm. (theoretical yield, assuming the anhydrous salt form, is 429.2 Gm.). The monosodium salt was then transferred to a 1-liter beaker, 500 ml. 95% ethyl alcohol and 294 ml. concentrated HCl (3.5 moles) were added and the mixture warmed at 50–60° for two hours before removal of the sodium chloride by filtration. The filtrate was evaporated to dryness with a subsequent yield of 200 Gm. crude *p*-chlorobenzenesulfonic acid. This product after dissolving in 100 ml. glacial acetic acid, filtering, cooling, and adding 175 ml. $CHCl_3$ gave 160 Gm. or a 41.5% theoretical yield of pure acid (dried in a desiccator over KOH pellets, and concentrated H_2SO_4) which subsequently melted at 93° (corr.) and checked with the revised m. p. of Tanasescu and Macarovici (17). Moreover, a test portion of the acid was converted into the sulfonchloride and the latter into the amide and the observed melting points of both derivatives were found in agreement with the accepted literature values. Strangely enough, even though the revised melting point for the acid (17) was first announced in 1938, the incorrect value of 68° (first reported by Krafft and Wilke (10) in 1900), which is the accepted m.p. value for the hydrate, still persists in such reference sources as Heilbron (8), Kamm (9), Shriner and Fuson (14), and Cheronis and Entrikin (3) although all such sources were printed since 1938. Suter (16) neither cites the work of Tanasescu and Macarovici as a reference, nor does he give either m. p. value for *p*-chlorobenzenesulfonic acid or its monohydrate.

CONDENSATION STUDIES

Three separate experimental attempts were undertaken to condense chloral with *p*-chlorobenzenesulfonic acid. The first and second runs were made under conditions simulating those meagerly described by Giral and Rangel (7). Thus, 0.2 mole of *p*-chlorobenzenesulfonic acid and 0.1 mole of technical chloral were mixed without any perceptible heat effect. After heating the mixture under reflux for three hours on a water bath it was poured into 300 Gm. crushed ice. The reaction mixture was completely miscible and thus the solution was evaporated to dryness. A strong odor of chloral was observed during the evaporation and the grayish residue was dissolved in 100 ml. water and poured into 300 ml. chilled saturated NaCl solution with the resultant precipitation of *p*-chlorobenzenesodium sulfonate which after filtering and drying, weighed 38.5 Gm. (0.18 mole), a recovery of 90% of the original *p*-chlorobenzenesulfonic acid used in the experiment.

A parallel run in which the same quantities of reactants were employed (except in this case the chloral was freshly prepared by dehydration of chloral hydrate with concentrated H_2SO_4 overnight) yielded under similar conditions, a water-insoluble fraction when poured onto ice. After filtration, the filter cake was suspended in a solution of 25 ml. of ethanol and 100 ml. water, and after refiltering and drying in air, weighed 8 Gm. This material on a block melting point determination indicated it was not DDT by softening at 135° and melting with slow decomposition at 145–170° with the simultaneous liberation of a pronounced odor of chloral. From its behavior it was believed to be a water-insoluble chloral-polymer. The addition of 300 ml. chilled saturated NaCl solution to the filtrate from the original water-insoluble crude filter cake yielded 41.5 Gm. dry sodium *p*-chlorobenzenesulfonate (theoretical weight equivalent to 0.2 mol. *p*-chlorobenzenesulfonic acid = 42.9 Gm.).

In a third run *p*-chlorobenzenesulfonic acid was first prepared as previously described for the synthesis of the acid, and then without its separation from the reaction mixture, treated subsequently with chloral in order to further establish whether DDT could be synthesized as claimed by Giral and Rangel. Thus 1 mole chlorobenzene and 1 mole of chlorosulfonic acid were reacted and then 0.5 mole of technical chloral added dropwise over a twenty-minute interval at a temperature of 10°. Stirring was continued for four hours and the reaction product allowed to stand overnight at room temperature. It was then poured into crushed ice and after filtration and drying, 91 Gm. of crude water-insoluble filter cake was obtained. The latter was washed alternately by decantation with 150 ml. cold ethyl alcohol and 150 ml. cold petroleum ether and after the second filtration, 49 Gm. of insoluble fraction remained. The latter, upon two successive recrystallizations from a mixture of $CHCl_3$ and petroleum ether was identified by its melting point of 145° as *bis*-(*p*-chlorophenyl) sulfone. The original acid filtrate from the water-insoluble reaction product upon treatment with chilled sodium chloride solution as previously described yielded 120 Gm. air-dried *p*-chlorobenzene sodium sulfonate. On the basis of the 49 Gm. crude sulfone (0.183 mole) or an equivalent 0.366 mole original chlorobenzene and 120 Gm. sodium *p*-chlorobenzenesulfonate (0.558 mole) a total of approximately 93% of the original chlorobenzene was accounted for. Thus, from the three runs described above there is no evidence for the formation of DDT from *p*-chlorobenzenesulfonic acid as claimed by Giral and Rangel (7). Since it has been demonstrated that DDT can be prepared by the addition of chlorosulfonic acid to a mixture of chlorobenzene and either chloral or chloral hydrate (4), and since it has also been shown that mono and dialkylated derivatives of aromatic hydrocarbons can be synthesized in a similar manner from the corresponding alcohols and appropriate aromatic hydrocarbons (13), it is obvious that the postulated mechanism of Rueggeberg (13) rather than that of Giral and Rangel (7) is in accord with all the experimental evidence presented for the synthesis of DDT.

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Mentha Cardiaca. I. Pharmacognostical Studies and a Preliminary Investigation of the Volatile Oil*

By KEITH D. BALLENTINE and ARTHUR E. SCHWARTING

A report of the histological, morphological, and growth characteristics of *Mentha cardiaca*. Chemical and physical constants of the oil are presented and comparisons with spearmint and its oil are made.

THE PLANT, *Mentha cardiaca* Gerarde, commonly called Scotch Mint and Little Leaf Mint, is a perennial *Labiatae* resembling *Mentha spicata* in structure and odor. The fact that it was observed to produce a volatile oil in a high percentage and that the volatile oil resembles spearmint oil prompted the present study. The available literature disclosed no chemical or histological investigation of the plant.

The plant is tall and erect with pubescent stems and leaves. The leaves are petiolate, unequally serrate, oblong-to-ovate-lanceolate with an acuminate apex. The leaf color is somewhat lighter green than that of spearmint, and the purple color which often appears on the basal stem of spearmint is not observed on *M. cardiaca*. The flowers exist in interrupted whorls in the axils of the leaves, the corolla being pale purple in color. The flowering dates parallel those of *M. spicata*.

EXPERIMENTAL

Cultivation.—Root cuttings were introduced to the medicinal plant garden in 1944. The area was expanded in 1945 and distillations were made in that year and in the following year from an area 50 feet square. In the spring of 1947 a systematic investigation of this and three other mints was begun and two areas of cultivation were established; the one a typical dry land area, and the other a fertile lowland with irrigation facilities. The main observations and data presented in this paper were obtained from these latter plantings. Four randomized plots of five 18-foot rows each of *Mentha cardiaca* appeared in each area. The rows were planted two feet apart, the single cuttings being planted one foot apart in the row.

The plant demonstrated unusual vigor, the root system expanding more rapidly than spearmint or peppermint. The crop was taken in the last week in July and at that time it was already difficult to outline single rows. A 16-foot length of the center row of each plot was collected and the weight recorded. These data were used in the preparation of Table I. The remaining plot area was also harvested and the total material of the four plots of each area was separately distilled. Because no significant difference regarding plant or oil yield from the plots of the two areas was observed the data appear as an average of all materials.

Distillation.—Three hundred forty pounds of the overground flowering plant was distilled in lots of about 50 pounds each. The plants collected in mid-morning were allowed to wilt in the field and distillation was initiated immediately and continued until all material was distilled. The aqueous waters were subjected to cohobation processes in a separate still and the oil collected. The oils were not mixed. Each was dried, filtered, and stored in tightly stoppered and filled containers. The yield, expressed as per cent, in Table I is the total oil.

TABLE I.—YIELD DATA OF *Mentha cardiaca*

Fresh Plant per Acre, Lbs. (Calculated)	Oil Content, %	Oil Yield per Acre, Lbs. (Calculated)
12,378	0.477	59.0

Leaf Histology.—Leaf and stem material was collected and fixed in a formalin–acetic acid–alcohol solution. A specimen of *M. spicata* was similarly prepared for a comparison of structures. The materials were embedded in paraffin and sectioned with a rotary microtome. Finished permanent slides were prepared of all specimens. Hand sections of these and fresh materials were also prepared.

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The lamina (Figs. 1 and 2) is thin and consists of the epidermal layers, the chlorenchyma, consisting of a single row of irregular palisade cells and a spongy region of three or four layers of cells. Both glandular and nonglandular trichomes occur on the leaf surfaces, being more numerous on the dorsal surface and on the veins. The uniseriate multicellular trichomes vary in length from 85 to 180 microns. Numerous single-celled trichomes occur. Glandular trichomes of the stalked type are numerous. The stalk is one or two celled with a globular gland composed of one cell up to 24 microns in diameter. Sessile glands are less common and are found in epidermal depressions. The gland is six to eight celled.

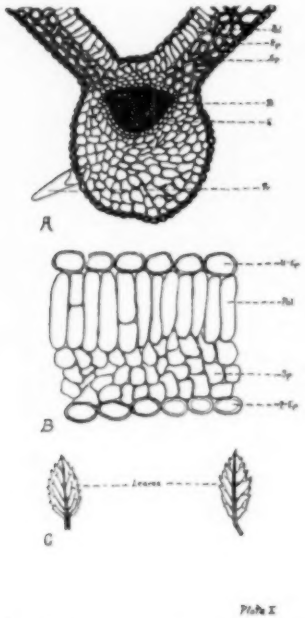

Fig. 1.—*Mentha cardiaca* Gerarde. T. S. of leaf. *A*, midvein; *B*, lamina. *U-Ep*, upper epidermis; *L-Ep*, lower epidermis; *Pal*, palisade cells; *Sp*, spongy parenchyma; *B*, fibrovascular bundle; *S*, sieve; C, leaves.

A peculiar epidermal structure is illustrated in Fig. 2 (*D*). It appears as an epidermal eruption and is surmounted by two uniseriate trichomes. The structure is not common.

Stomata are more numerous in the dorsal epidermis of the lamina and are somewhat larger in the ventral surface. Two or three contiguous cells surround the guard cells and occasionally the stomata appear capped as illustrated in Fig. 2 (*E*).

Stem Histology.—The stem (Fig. 3) is an amphiphloic siphonstele structure, typical of the *Labiatae*. A cuticle covers the epidermal cells and numerous uniseriate trichomes and a few glandular trichomes

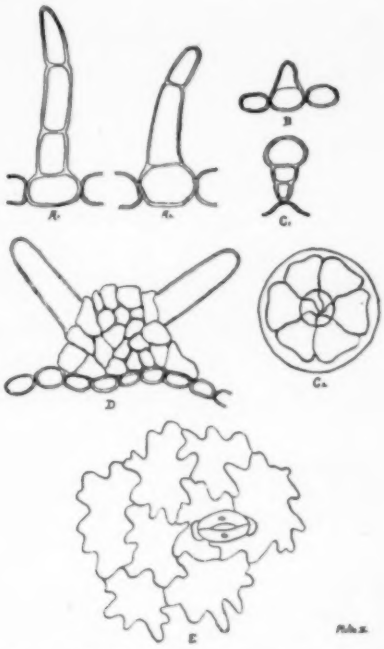

Fig. 2.—*Mentha cardiaca* Gerarde. Epidermal structures. A_1, A_2, *B*, uniseriate nonglandular trichomes; C_1, C_2, glandular trichomes; *D*, epidermal structure; *E*, lower epidermis of lamina showing stomata.

occur. The former measure up to 200 microns in length and are composed of as many as 7 cells.

A comparison of these structures in a typical specimen of *M. spicata* demonstrates several differences. Cardiaca trichomes of the nonglandular type are longer and the apical cell is more rounded than in *M. spicata*. The length:width ratio of *M. cardiaca* leaf is 1.85 while that ratio of *M. spicata* is 2.5.

The Volatile Oil

The volatile oil was collected in two portions: the oil, separated by distillation, and the cohobated oil. The oil was a red-brown oil with a sweet spearmint-like odor. The cohobated oil was amber colored and possessed a similar odor. The latter oil made up 8% of the total oil yield.

Physical constants.—Oil: Sp. gr. $\frac{25}{25}$ 0.9128; $[\alpha]_D^{25}$ −69.85; n_D^{20} 1.4871. Cohobated Oil: Sp. gr. $\frac{25}{25}$ 0.944; $[\alpha]_D^{25}$ −56.91; n_D^{20} 1.4925.

Ketones.—The neutral sulfite method (1) gave 57% average for the oil and 84% average for the cohobated oil. The absorption product of each was decomposed and the oils collected by steam distillation. Found: n_D^{20} 1.498, corresponding to carvone, for each oil. The oxime prepared from each oil had a melting point of 72°, corresponding to carvone.

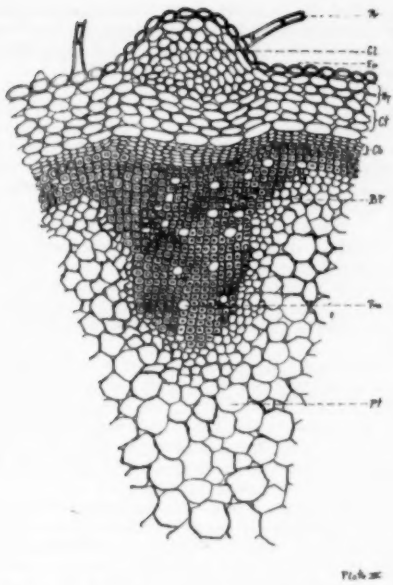

Fig. 3.—*Mentha cardiaca* Gerarde. T. S. of stem. *Tr*, trichome; *Cl*, collenchyma; *Ep*, epidermis; *Hy*, hypodermis; *Cl*, cortical parenchyma; *Cb*, cambium; *Bf*, fibers; *Tra*, trachea; *Pt*, pith.

Acid Number.—The method of Guenther (2) gave an average of 0.556 for the oil and 1.005 for the cohobated oil.

Esters.—The ester number for the oils was determined according to the method of Guenther (3). Results were variable. The results of eight determinations with each oil gave values between 12.64 and 19.79 for the oil and between 14.73 and 23.68 for the cohobated oil. An average of 16.215 for the oil corresponds to 5.6% of linalyl acetate. A similar calculation gave 6.7% for the cohobated oil.

Alcohols.—The ester number after acetylation (4) was 28.82 for the oil and 42.97 for the cohobated oil. By using the average ester values and these results, the free alcohol, calculated as linalool, was found to be 3.5% in the oil and 6.6% in the cohobated oil.

Phenols.—The general procedure of Guenther (5) demonstrated an absence of phenolic bodies in both oils.

SUMMARY

A histological study of the leaf and stem of *Mentha cardiaca* Gerarde has been presented. A comparison with *M. spicata*, of typical structures, was made.

The growth characteristics, crop yield data, and oil yield of a one-year-old planting have been recorded.

A partial chemical investigation of the volatile oil has been made. Data are reported for certain physical and chemical constants. The distilled oils contain a high ketone content, the identity of which is carvone.

Since this is a preliminary investigation of the volatile oil of *Mentha cardiaca* Gerarde, additional investigation will be necessary before a complete report can be made.

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The Properties of the Oil of *Xanthium Spinosum**

By C. JELLEFF CARR

Some chemical and physical properties of the oil obtained from *Xanthium spinosum* are reported. Preliminary studies on toxicity and on blood pressure response indicate that the oil is pharmacodynamically inert.

XANTHIUM, cocklebur or spiny clotbur is a member of the *Compositæ* family naturalized in this country from Europe. The burs of the plant have been employed in the preparation of a tea infusion for use by mouth in the treatment of arthralgias. Clinical and pharmacologic investigations have been conducted with extracts of this plant. Previously, the chemistry and pharmacology of *Xanthium spinosum* was reported in THIS JOURNAL (1). Choline was isolated from aqueous extracts prepared from the burs of the plant by forming the readily crystallizable perchlorate salt of the nitrate ester (2). During the preparation of aqueous extracts of

* Received October 12, 1948, from the Department of Pharmacology, School of Medicine, University of Maryland, Baltimore, Md.

xanthium burs it was observed that an oil separated from the aqueous concentrates. Subsequently the burs were rendered fat-free by repeated extraction with petroleum ether. The pale yellow oil obtained in this manner was subjected to a chemical and pharmacologic examination.

A glycoside and an unidentified active principle have been obtained from *Xanthium strumarium* (3), and Maksimov obtained an oil from the seeds of this plant and described its physical and chemical properties as resembling those of sunflower-seed oil (4).

The burs used in this investigation were identified pharmacognostically as *Xanthium spinosum* by Professor Heber W. Youngken. The oil was obtained from burs from plants grown in Iowa, Maryland, and Ohio.

EXPERIMENTAL

Isolation of the Oil.—The air-dried, coarsely ground burs were extracted in a percolator with petroleum ether until the drug was exhausted. The percolate was collected and the petroleum ether recovered by distillation on a steam bath. A light yellow oil was recovered. The average oil yield was 7.9% of the weight of the air-dried burs. A fine crystalline material separated from the oil, which was identified as stearin (m. p. 65°) and an unidentified wax (m. p. 83°). The oil was dried for one month in a desiccator under a vacuum at room temperature and subjected to the following examination.

Description of the Oil.—The dry, clear, light yellow oil obtained by the method previously described exhibits a peculiar characteristic odor. It is tasteless and burns with a smoky flame and with a typical fatty odor. The oil is acid to moist litmus paper. It is insoluble in alcohol and slightly soluble in boiling alcohol. Xanthium oil is very soluble in chloroform and acetone, and soluble to a limited degree in isopropanol. It is slightly soluble in boiling water.

Three oils prepared from different samples of the drug, from which the stearin had been removed, gave the following refractometer readings: n_D^{28} 1.4750, n_D^{25} 1.4755, and n_D^{25} 1.4755. The specific gravity at 28° was 0.920.

Xanthium oil when viewed in an ultraviolet light with a Wood's filter exhibits a strong purple-blue fluorescence differing from cottonseed oil, which exhibits a lighter blue fluorescence.

Xanthium oil prepared from different samples of the burs gave the following values for fats and fatty oils as determined by the methods described in United States Pharmacopœia XIII. The free fatty acids in xanthium oil gave an acid value of 20.2 cc. of 0.1 *N* alkali required to neutralize the free acid in 10 Gm. of the oil. The saponification value was found to be approximately 200. An average ester value of 188 was obtained with an iodine value of 130. A light yellow wax constituting approximately 3.5% of the oil was obtained as unsaponifiable matter. This wax became hard and brittle upon aging. The soap prepared from the saponified oil possessed good cleansing properties. The esters of the fatty acids present in the oil are glyceryl esters. A satisfactory yield of glycerin was obtained from one sample of the oil.

After separation of the stearin and wax by cooling, the oil was distilled under a reduced pressure of approximately 30 mm. Hg. A trace of a volatile fraction was obtained between 20–100° and approximately equal volumes of three fractions distilling between 100–240° and 240–360°, and a heavy oil residue that does not solidify at room temperature. There was very little decomposition of the oil during distillation at these temperatures. Samples of the oil exposed in a desiccator to sunlight and air for six years have retained their pale yellow color and have not developed a rancid odor. A thin film of gum formed upon the surface of the oil.

The mixed fatty acids prepared from the oil according to the U. S. P. method are dark brown in color and do not solidify at room temperature. At 15° the liquid mixed fatty acids begin to solidify and at 5° are almost completely solid. For this reason not more than traces of palmitic or stearic acids are believed to be present.

Miscellaneous Tests.—An examination of the oil for vitamin A by the Carr-Price reaction gave a negative test. The U. S. P. identity test for cottonseed oil with a solution of sulfur in carbon disulfide and amyl alcohol yields with xanthium oil a brown color in place of the brilliant red of cottonseed oil. A negative test for resin oils was obtained. Baudouin's test yields a deep red color with xanthium oil. The color resembles that produced by sesame oil.

The oil was found to be neither attractive nor repelling to a variety of insects when applied to the skin and when substituted for the oil employed as a lure in insect traps.

Pharmacodynamic Activity.—In experiments to determine the nature of the depressor substance in xanthium the following studies were made. The intravenous injection in the dog of from 2 to 5 cc. of physiological salt solution saturated with xanthium oil did not significantly influence the blood pressure. The injection of from 2 to 5 cc. of a 10% solution of the polyols obtained from the saponification of the oil produced a fall in blood pressure of from 10 to 22 mm. of Hg. (The injection of an equivalent dose of glycerin was without effect upon the blood pressure.) The saponified oil, upon intravenous injection of 2 cc. of a 5% solution produced a fall in blood pressure equivalent to that elicited by an equivalent dose of sodium oleate. Saturated aqueous solutions of the mixed fatty acids obtained from xanthium oil were without effect upon the blood pressure when injected intravenously. A 5% emulsion of the oil upon intravenous injection elicited no depressor response.

The feeding of a diet containing 1% of xanthium oil to white rats for a period of 6 weeks produced no visible toxic effects. The animals gained weight at a normal rate. The oral administration of doses of from 1 to 2 cc. of xanthium oil by stomach tube to white rats produced only a mild degree of catharsis but no toxic symptoms during an observation period of two days.

From the animal experiments it appears that the fixed oil obtained from *Xanthium spinosum* is

relatively pharmacologically inert and probably does not represent a therapeutically active portion of the plant.

SUMMARY

The properties of the oil obtained from the burs of *Xanthium spinosum* have been described. Preliminary studies on blood pressure response and on toxicity indicate that the oil is pharmacodynamically inert.

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Radio-Frequency Drying of Tablet Granulations*

By H. BIKIN, GLENN L. JENKINS and H. GEORGE DeKAY

This project was undertaken to survey the possibilities of utilizing radio-frequency drying of tablet granulations for future work of a more extensive nature. Two possible theories concerning the actual mechanism of nonconductor materials in a radio-frequency field are reviewed explaining how the nonconductor material becomes heated from within rather than by application of heat to its perimeters and the subsequent transfer of the heat to the center. A number of important electrical considerations regarding the size and materials of construction of the electrodes and their positions relative to one another are discussed. A table of thirty-one chemicals, with the percentage of added water removed, the period of drying time, and the peak radio-frequency voltage used, is given.

THE PROCESS of manufacturing tablets can be broken down roughly into the following steps: weighing, mixing, granulating, drying, and compressing. If the drying operation, which is very time-consuming, could be handled by radio-frequency radiation, the manufacturing cycle could be multiplied by a factor and a continuous production line would become feasible.

Drying consists of two phases: the separation of the media to be eliminated from the material to be dried, and the actual removal of the separated media from the material. Only the first phase can be accelerated to give an appreciable decrease in over-all drying time by increasing the amount of heat applied to the material during the drying operation.

Radio-frequency radiation drying makes use of the heating effect caused in nonconductor materials by the rapid alternations of the radio-frequency field. In addition to placing the material between two parallel plate electrodes to effect drying, it is also possible to use the stray field method in which the electrodes consist of a series of parallel rods or tubes over which the material passes. Alternate electrodes are connected together with one set tied to the high voltage source and the other set to the ground lead to form an electrical field of an ellipsoidal shape between them. At present, either of the two following concepts, concerning what actually takes place in a material which is placed in a radio-frequency field, are acceptable.

According to Duryee (1), each substance is composed of a positively charged nucleus around which negative electrons revolve in fixed orbits with the number of orbits dependent upon the atomic structure of the substance. As this substance is subjected to a high-voltage, high-frequency field, the orbits of electron travel are severely stressed and cause molecular friction. This friction results in heat generation and the whole mass of the substance becomes uniformly heated from within.

The explanation given by Venable (2) states that with no external electrical field present, a molecule might be seen to have its centers of positive and negative charges coincident, or these centers may be displaced relative to one another. As the external electrical field changes, the molecule described in the former instance might be seen to have its coincident centers displaced relative to one another forming individual dipoles whose axes tend to parallel the electrical field. The molecule described in the latter instance is called a permanent molecular dipole and it too

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will tend to rotate until its axis is parallel to the electrical field. In some cases there also may be an added displacement of the electrical centers of the permanent dipoles. Work is done by the displacement of the electrical centers of the molecules, by the rotation of the dipoles, or by both.

Materials which are being dried do not begin to heat up excessively until the point of latent heat of vaporization has been passed, and if, at that time, they can instantly be removed from the drying chamber there will be no perceptible rise in temperature (3). In ordinary drying methods this situation is very difficult to control because the heat is first applied to the perimeters of the material and then is transferred to the center. In radio-frequency drying the heat is generated within the substance so that the entire mass is uniformly heated and additional heat can be discontinued at any second by throwing a switch and breaking a circuit.

Actual experimental work with a radio-frequency drying unit must be preceded by a correlation of the limiting electrical requirements of this new tool. To obtain a uniform field, the electrodes must necessarily be equidistant at all points. Large electrodes may cause nonuniform heating due to standing waves (4), but this may be overcome by "multiple tuning"[1] or by reducing the dimensions of the electrodes to less than one-eighth of a wave length of the frequency being used (5). Brass, steel, copper, or aluminum electrodes show no appreciable differences although aluminum may be preferred because of its weight and its resistivity to corrosion. All sharp corners should be removed in order to prevent corona arcing and to increase the amount of permissible voltage to be applied. The dimensions of the parallel plate electrodes should exceed those of the material placed in the radio-frequency field to keep the material out of the uneven, fringed field which forms around the edges of the electrodes. To prevent the moisture given off by the granules from condensing upon the upper electrodes and to aid in the removal of the moisture separated from the granules, a small fan is useful.

Each of the chemicals in Table I was mixed with the amount of distilled water deemed necessary to obtain a granule when the material was passed through a 30-mesh sieve. The percentage of moisture lost was based upon the amount of water added to the granulation.

TABLE I.—DRYING OF CHEMICALS

Chemical	Grams Used	Grams of H_2O Added	Kv. (Peak R.F.)	Time, Min.	% H_2O Removed
Aspirin	194.0	31.0	8.0	15	95.3
Starch	117.1	30.4	10.4	30	84.0
Sulfanilamide	133.5	35.5	8.5	30	100.0
Acetanilid	137.1	21.9	8.75	45	89.2
Acetophenetidin	178.4	28.6	8.5	45	91.0
Sodium Chloride	253.5	13.4	12.5	30	100.5
Sucrose	183.2	9.3	11.0	30	102.2
Calcium Gluconate	94.1	26.4	8.5	30	96.8
Calcium Carbonate	144.0	48.0	8.5	45	95.8
Magnesium Carbonate	72.5	79.5	8.0	30	105.0
Dextrin	128.0	20.5	9.0	30	98.5
Ammonium Chloride	128.6	45.9	13.0	135	94.0
Sulfathiazole	162.4	21.1	8.0	30	94.7
Sodium Nitrate	324.5	8.5	9.0	45	71.0
Sodium Borate	130.8	32.7	9.0	45	98.0
Tribasic Sodium Phosphate	102.5	7.0	9.5	45	107.0
Calcium Sulfate	165.2	48.8	8.0	30	74.0
Tartaric Acid	128.9	8.6	9.0	15	93.0
Tribasic Magnesium Phosphate	42.0	100.0	9.5	105	73.0
Magnesium Trisilicate	124.0	31.0	8.0	15	111.0
Tribasic Calcium Phosphate	213.1	25.4	8.0	15	97.8
Dicalcium Phosphate	221.0	26.5	8.0	15	98.0
Sodium Acid Phosphate	185.6	22.4	12.0	45	40.1
Magnesium Oxide	98.3	59.7	8.0	45	80.2
Dextrose	175.2	10.8	12.0	60	102.0
Calcium Lactate	158.3	31.7	6.5	45	91.5
Sodium Sulfate	218.9	18.6	9.0	45	89.0
Methenamine	146.2	12.4	9.0	45	80.6
Aminopyrine	161.3	28.2	8.0	45	89.0
Bismuth Subcarbonate	147.5	34.0	13.0	45	95.7
Phenobarbital	123.8	40.7	8.5	30	95.7

[1] R. A. Bierwirth, U. S. Patent 2308043 (1943).

While aspirin, dried by radio-frequency heating, does hydrolize to some extent as evidenced by the pronounced odor of acetic acid, nevertheless the free salicylic acid test of the United States Pharmacopœia is not exceeded.

Sodium bicarbonate loses over ninety per cent of the added moisture when dried in this manner but it decomposes so that the carbonate test of the United States Pharmacopœia is exceeded.

Of outstanding importance is the fact that a good number of widely used chemicals and tablet excipients can be dried rapidly and easily.

SUMMARY

Of the thirty-one chemicals chosen, twenty-seven were dried of eighty per cent or more of the added moisture in a satisfactory manner.

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Infrared Drying of Tablet Granulations. I*

By B. N. PATEL, GLENN L. JENKINS and H. GEORGE DeKAY

Recent improvements in the manufacture of compressed tablets have been outlined and the need for a rapid and continuous drying procedure for tablet granulations indicated. Infrared drying equipment is discussed and the possible advantages of such procedures are presented. The results of the experiments carried out in this work lead to the conclusion that infrared drying is rapid and uniform and could be used advantageously in drying tablet granulations.

NUMEROUS medicinal agents have been administered in the form of compressed tablets for the past few decades. It has been estimated that close to one-third of the medication used today is administered in this form.

The manufacture of compressed tablets involves four important steps: the weighing of the ingredients, the granulating, the drying, and the compressing of the dried granulation into desired shapes of tablets. Much progress has been made in the field of tablet manufacture in recent years through the use of perfected machinery. Mixing, granulating, and compressing have been improved to such an extent that large quantities can be processed in relatively short periods of time.

The operation of drying the granulation has remained practically unchanged and it requires at present as much time, if not more, as the weighing, granulating, and compressing combined. Many types of drying closets are used. For years (1) the most popular was the ordinary closet, arranged in series, heated by steam coils in the back, and having an opening near the base and an opening at the top for the escape of evaporated moisture. Later came developments involving the use of the air in outside chambers and the uniform distribution of the hot air by means of properly arranged flues (1).

Cook and LaWall (2) state that a number of laboratories manufacturing a line of tablets are provided with special equipment which automatically controls the humidity and temperature of the whole laboratory space in which the processes of tabletting are executed. This points out how essential proper drying facilities are considered to be by experienced tablet makers.

According to Chilson (5), drying signifies a change in the vapor pressure of the moisture contained in a solid. Since this change must be produced by heat, all apparatus designed for drying or dehydration is simply a means of conveying heat to the moisture contained in a solid. Thus to shorten the time needed to dry tablet granulations, devices must be found which will transfer more heat to the moisture in the granulation than the dry warm air or an electric oven, and which will promote more uniform heating.

Victor (4) states that good results have been obtained by using low drying temperatures in an oven with controlled humidity. The majority of granulations can be dried efficiently to the required final moisture content at relatively high humidities and at moderate temperatures.

The trend in the use of tablets has been one of steady increase. The National Formulary pioneered in the establishment of official standards for tablets. Tablets increased (6) virtually

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from none in 1920 to 17 per cent of all the official admissions of dosage forms in 1945. This expansion in the field of the manufacture of compressed tablets calls for a method or methods whereby the drying time could be sufficiently shortened. It should also be essential to design equipment for a continuous process which would take at most not more than two or three hours from the time the powders were weighed until the first tablets were compressed.

The various methods (5) of drying are: (1) convection drying (2) radiation drying, (3) conduction drying, (4) induction drying, (5) electronic drying, (6) resistance drying, (7) absorption drying, and (8) sublimation drying. Of the various ways of applying heat to an object, radiation is recognized as the most efficient, provided the path that the radiant energy has to travel can be kept short (3). It is characteristic of this type of drying that heat will be radiated from a heat source regardless of interposed air. It is, therefore, the object of this research project to investigate the possibility of using heat radiations produced by infrared lamps to shorten the time required to dry tablet granulations without adverse effects on the materials treated.

EXPERIMENTAL

Lamps encased in a metal casing as supplied by The Fostoria Pressed Steel Corporation, Fostoria, Ohio, were mounted on a wooden rack in a stable position. The wooden rack was supported by screwed supports which can be replaced by shorter or longer supports as required to decrease or increase the distance between the material heated and the source of heat. The material to be dried was placed in a suitable container directly below the lamps.

A. Heating of Chemicals.—To determine whether the heat from the lamps would cause any decomposition of chemicals commonly used in tablet making, a number of chemicals were heated in 5-Gm. quantities in Petri dishes or paper dishes for twenty minutes under 250-watt, 375-watt (blue), and 500-watt bulbs at distances of 1 foot and 2 feet. Visual physical changes, if any, were noted and changes in weight determined.

The results of the experiment are depicted in Table I.

It will be observed from the table that a majority of the substances do not undergo any changes when thus heated under lamps. The temperature reached during twenty minutes' heating ranged from 70° to 75° in the case of the 250-watt white bulb, 95° to 115° in the case of the 375-watt blue bulb, and 95° to 100° in the case of the 500-watt white bulb when the distance was 1 foot. Corresponding temperatures at a distance of 2 feet are 45° to 50°, 83° to 85°, and 55° to 58°.

B. Drying Tablet Granulations.—Various tablet granulations prepared according to known formulas using starch paste as a granulating agent, unless otherwise stated, were dried under different lamps at a distance of 22 inches and with a layer thickness of 1/4 inch. (The starch paste used was prepared as follows: mix one part of corn starch with three parts of cold water in a beaker, place the mixture in a boiling water bath and stir until the paste begins to thicken. Remove the beaker and cool in cold water with continuous stirring.) The dried granulations were compressed after proper lubrication. The results of this experiment are given in Table II.

C. Prolonged Heating of Chemicals.—In order to ascertain the effects of prolonged heating by infrared lamps on chemicals, a few of the chemicals used in granulations during this work were heated for two hours under 375-watt and 500-watt lamps, keeping the distance at 22 inches; then the loss in weight was determined.

Among the chemicals thus treated, the following showed no apparent changes: acetanilid, aspirin, aminopyrine, calcium lactate, calcium phosphate, lactose, phenacetin, potassium bicarbonate, potassium bromide, sulfanilamide, sulfathiazole, sodium benzoate, and sodium salicylate. Amytal, bismuth subnitrate, caffeine citrate, lithium citrate, and sulfapyridine showed slight loss in weight while magnesium trisilicate showed considerable loss in weight when heated under a 500-watt bulb and benzocaine underwent decomposition under 375 watt and 500-watt bulbs.

CONCLUSIONS

From the work done under the present project the following conclusions were drawn:

1. Heating by infrared lamps was rapid and the heat distribution was uniform throughout. Best results were obtained with a 375-watt white bulb at a distance of 22 inches. The 375-watt blue bulb caused decomposition in many cases. It was not clear whether the chemicals which were designated as light-sensitive (of course, to different extents) were affected by the infrared light or not.

2. Rate of drying depends, as expected, on the moisture content of the material and the thickness of the bed of granules. Thickness of 1/4 inch gave better results than thickness of 1/2 inch.

3. Use of radiant heating by infrared lamps on an industrial scale can be more economical as compared to air-drying ovens since, in the latter case, it is necessary in big factories to maintain the oven constantly at heat in readiness for work at all times. Further, it is necessary that the temperature be brought to a peak in the ovens long enough before they are to be used so that they will be ready to dry the material put into them. For the same reasons, a substantial amount of stored heat is lost at the end of the day if the ovens are maintained on an eight- or sixteen-hour cycle. With the infrared equipment it is not necessary to turn on the light until the granulation is actually ready for drying.

TABLE I.—EFFECTS OF INFRARED HEAT ON CHEMICALS

No.	Material	250-Watt White Bulb 1 Ft.	250-Watt White Bulb 2 Ft.	375-Watt Blue Bulb 1 Ft.	375-Watt Blue Bulb 2 Ft.	500-Watt White Bulb 1 Ft.	500-Watt White Bulb 2 Ft.
1	Acetanilide	...	NC	...	LD	...	NC
2	Aloin	...	NC	...	B	...	SL
3	Aminopyrine	NC	NC	LD	NC	LD	NC
4	Ammonium Bromide	...	NC	...	NC	...	NC
5	Ammonium Chloride	...	NC	...	NC	...	NC
6	Amytal	...	NC	...	NC	...	NC
7	Arsenic Trioxide	...	NC	...	NC	...	SL
8	Ascorbic Acid	NC	NC	B	B	NC	NC
9	Benzocaine	D	NC	LD	LD	LD	NC
10	Benzoic Acid	...	NC	...	LD	...	D
11	Bismuth Subcarbonate	...	NC	...	NC	...	NC
12	Bismuth Subgallate	...	NC	...	SL	...	NC
13	Bismuth Subnitrate	...	NC	...	SL	...	NC
14	Borax	...	NC	...	NC	...	NC
15	Boric Acid	...	NC	...	SL	...	NC
16	Caffeine	NC	NC	NC	NC	SL	NC
17	Caffeine Citrate	...	NC	...	NC	...	NC
18	Calcium Bromide	...	NC	...	NC	...	NC
19	Calcium Carbonate	...	NC	...	NC	...	NC
20	Calcium Chloride	...	W	...	W	...	W
21	Calcium Gluconate	NC	NC	NC	NC	NC	NC
22	Calcium Glycerophosphate	...	NC	...	SL	...	NC
23	Calcium Lactate	NC	NC	SL	SL	SL	SL
24	Calcium Phosphate (dibasic)	...	NC	...	NC	...	NC
25	Calcium Sulfide	...	NC	...	NC	...	NC
26	Camphor (monobromated)	...	NC	...	LD	...	LD
27	Cerium Oxalate	...	NC	...	SL	...	NC
28	Chloramine T	...	D	...	D	...	D
29	Cincophen	...	NC	...	NC	...	NC
30	Dextrin	NC	SL	SL	SL	SL	SL
31	Dextrose	...	NC	...	NC	...	NC
32	Ephedrine (Alkaloid)	LD	LD	LD	LD	LD	LD
33	Ephedrine Sulfate	NC	NC	NC	NC	NC	NC
34	Ferrous Sulfate (dried)	...	NC	...	NC	...	NC
35	Gelatin	SL	SL	SL	SL	SL	SL
36	Lactose	NC	NC	NC	NC	NC	NC
37	Lithium Citrate	...	NC	...	SL	...	NC
38	Magnesium Carbonate	...	NC	...	NC	...	SL
39	Magnesium Oxide (heavy)	...	SL	...	SL	...	NC
40	Magnesium Salicylate	...	NC	...	D	...	NC
41	Magnesium Sulfate	...	NC	...	D	...	NC
42	Magnesium Trisilicate	...	SL	...	SL	...	SL
43	Mercuric Iodide	...	NC	...	SL	...	SL
44	Mercurous Chloride	...	NC	...	W	...	NC
45	Mercurous Cyanide	...	NC	...	NC	...	NC
46	Methenamine	...	NC	...	NC	...	NC
47	Pepsin	...	SL	...	SL	...	SL
48	Phenacetin	NC	NC	D	NC	NC	NC
49	Phenolphthalein	...	NC	...	NC	...	NC
50	Potassium Bicarbonate	...	NC	...	NC	...	NC
51	Potassium Chloride	...	NC	...	NC	...	NC
52	Potassium Citrate	...	NC	...	NC	...	NC
53	Salicylic Acid	...	NC	...	D	...	NC
54	Salol	...	LD	...	LD	...	LD
55	Sodium Benzoate	...	SL	...	NC	...	SL
56	Sodium Bicarbonate	SL	NC	SL	NC	SL	NC
57	Sodium Bromide	...	NC	...	W	...	NC
58	Sodium Chloride	...	NC	...	NC	...	NC
59	Sodium Citrate	...	NC	...	NC	...	NC
60	Sodium Nitrate	...	NC	...	NC	...	NC
61	Sodium Salicylate	...	NC	...	NC	...	NC
62	Starch (potato)	SL	SL	SL	SL	SL	SL
63	Sulfanilamide	NC	NC	NC	NC	NC	NC
64	Sulfapyridine	NC	NC	NC	NC	NC	NC
65	Sulfathiazole	NC	NC	NC	NC	NC	NC
66	Tartar Emetic	...	NC	...	NC	...	NC
67	Zinc Phosphide	...	NC	...	NC	...	NC

The letters used in the table will be interpreted as follows: D = decomposition; W = mass becoming damp; SL = slight loss in weight; B = darkening; NC = no apparent change; LD = liquefaction and decomposition.

TABLE II.—COMPRESSION RESULTS ON DRIED GRANULATIONS

Granulation	Bulb,[a] Watt	Results of Drying	Time Required, Min.	Compression
Bismuth Subnitrate	250	Incomplete[b]	80	
Calcium Lactate	250	Dried	101	Satisfactory
Alkaline, Aromatic	500	Dried	40	Satisfactory
Aspirin Compound	500	Dried	40	Satisfactory
Alkaline, Effervescent	500	Dried	35	Satisfactory
Antiseptic	500	Decomposition		
Acetanilid & Caffeine Compound	250	Dried (Loss of Camphor)	70	Satisfactory
Acetanilid & Caffeine	500	Dried	40	Satisfactory
Sulfanilamide	375	Dried	55	Satisfactory
Sodium Bromide Compound	375	Dried	60	Satisfactory
Aminopyrine (Starch paste)	375	Decomposition		
Aminopyrine (Gelatin-acacia solution)	375	Dried	50	Capping
Sulfathiazole	375	Dried	50	Satisfactory
Mixed Treatment #2	375	Dried	80	Satisfactory
Phenacetin & Salol	250	Liquefied		
	375	Liquefied		
Benzocaine Compound	250	Dried	55	Satisfactory
	375	Dried	55	Satisfactory

[a] Two bulbs were used to dry the material. They were white bulbs.
[b] The wet granulation dried completely on 15 minutes' additional heating under two 500-watt bulbs.

4. There is a field for further study in the drying of tablet granulations by infrared lamps. Granulating equipment can be connected to a drying tunnel consisting of a movable belt or screen as a means of conveying the uniformly spread wet granules to be dried by the heat of the infrared lamps attached to the inner walls of the tunnel; thus granulation can be effected continuously. The tunnel can be constructed on the principles of the automobile industry which uses infrared lamps to bake paints on automobile bodies. The tunnels can be equipped so as to process conveniently small as well as large batches.

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Infrared Drying of Tablet Granulations. II*

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Twenty-two formulas containing inorganic compounds, nineteen formulas containing organic compounds, and fourteen compound formulas were granulated using the usual technique. The granules were dried under infrared lamps and compressed after lubrication. Favorable results were obtained in about ninety per cent of the cases. Temperature and period of drying in a majority of the cases were below 60° and 25 minutes, respectively. Results of assays on the granulations showed complete absence of untoward effects of infrared heat.

THIS IS A continuation of the previous work (29) undertaken to investigate the possibility of using infrared lamps for successfully drying tablet granulations. The nature of the problem has been outlined in the previous paper.

HISTORY

In April, 1935, a patent (1) specifying the use of radiant energy of the wave length 10,000 Å. to 16,000 A. for baking enamel on automobile

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bodies was issued to F. J. Groven and assigned to the Ford Motor Company. Since then incandescent lamps and other equipment furnishing infrared rays have been used on an industrial scale for a variety of heating, baking, and drying operations. Examples of such applications are:

1. Drying paints, varnishes, and lacquers (4, 5, 8, 9, 10, 15, 17, 22).
2. Softening, expanding, or treating metal, cellulose, and plastic materials (6, 10, 11, 12, 13, 18).
3. Baking out radio coils and transformers (7).
4. Drying enamel and glazing solutions (20)
5. Drying in leather, latex, and textile industries (8, 20).
6. Drying chemicals and heating in carrying out chemical reactions (19, 27).
7. Dehydration and preservation of biologicals and food materials (15, 16, 28).
8. Evaporation and removal of water from commercial materials (10, 11, 14, 21, 24, 25).

Drying experiments on tablets made from aspirin, phenacetin and caffeine, phenobarbital tablets, mannitol hexanitrate tablets, and synthetic estrogen tablets have been successful (26).

Radiant heat has also been used for drying purposes in combination with other methods of drying (2, 23).

EXPERIMENTAL

The preliminary work (29) on drying wet tablet granulations by infrared lamps gave results that indicated the usefulness of further study in the same field.

It was then considered necessary to carry out this process of drying on a larger scale to simulate, to some extent, the conditions that prevail in commercial production. Considering the advantages of a conveyorized process over a stationary one in infrared heating operation, a belt conveyor was designed and built so as to secure movement of the granular material under the lamps at a speed which will permit uniform heating with resultant drying to a moisture content conducive to satisfactory compression.

A canvas belt is driven by a motor with desired gearing-down setups at a speed of 1 foot every three minutes. For feeding the granular material so as to have a uniform layer of known thickness, a removable wooden box, with an adjustable metal band to regulate the thickness of the layer of the granular material, was fitted over the feeding end of the belt. The two lamp units as supplied by the Fostoria Pressed Steel Corporation, Fostoria, Ohio, were suspended over the belt with a provision for raising or lowering to increase or decrease the distance between the source of heat and the material being treated. There are two and four lamps, respectively, in each of the lamp units. The capacities of the infrared lamps intended to be used in the process of drying are 250 watt, 375 watt, and 500 watt, respectively.

PART I

Drying and Compressing of Tablet Granulations.—With a view to determining the optimum conditions with regard to thickness of the bed of granular material (which will be referred to as the thickness hereafter), and distance between the source of heat and the material being treated (which will be referred to as the distance hereafter), the drying operation was carried out on 5-pound batches of sodium bicarbonate granulation. Sodium bicarbonate was selected for this purpose because of its low cost.

Sodium bicarbonate granulation prepared with starch paste as the granulating agent was fed on the belt through the feeding box adjusted to give a definite thickness and dried under 375-watt and 500-watt lamps with the distance kept at 15 inches or 22 inches. The granules carried over the belt under the lamps were collected in a receptacle at the discharge end. The time over which the granules were exposed to infrared heat (which will be referred to as the time hereafter) and the temperature reached at the end of the run (which will be referred to as the temperature hereafter) were noted and recorded. The temperature recorded was the temperature indicated by a thermometer kept for one minute in about 100 Gm. of the heated material collected by scraping across the width of the belt just before it reached the discharge end. The granules collected after the first run had to be subjected to a second equally long or short run under the lamps to dry them to the desired moisture content. Time and temperature were noted and recorded as in the previous run. The dried granulation was properly lubricated and then compressed. No difficulties were encountered during compression. The period of drying, the temperature, the thickness, and the distance were thirty-one minutes, 65°, 3/8 inch, and 15 inches, respectively.

Since lactose and powdered sucrose are very widely used in tablet formulations, lactose and powdered sucrose granulations prepared with starch paste were subjected to the drying operation in the same way as sodium bicarbonate granulation. It was found that lactose and sucrose granulations can be successfully dried by infrared heat in about seventeen to twenty-one minutes, the temperature remaining below 60°. The distance and thickness were 15 inches and 3/8 inch, respectively. These granulations compressed satisfactorily.

The results of drying experiments with sodium bicarbonate, lactose, and sucrose granulations indicate that a distance of 15 inches and a thickness of 3/8 inch could be satisfactory for successful drying, other conditions being more or less identical. A variety of tablet formulas were selected and granulations of the respective formulas were prepared by the use of a suitable granulating agent and were subjected to the drying operation under appropriate infrared lamps for the necessary period of time, keeping the thickness at 3/8 inch and distance at 15 inches in all cases. The temperature of the heated stock was taken as first described

after each run and the time for the entire drying operation was noted in each case. The dried granulation was lubricated properly and compressed. The results of this part of the experimental work are given in Tables I, II, and III.

The legends used in Tables I, II, and III will be interpreted as follows:

T = Talc	B = Binding
ST = Starch	STI = Sticking
MS = Magnesium Stearate	Del. = Substance deliquesces
LC = Less capping	VS = Very soft
P = Picking	SO = Soft

No. 1 = 2% Magnesium Stearate + 2% Starch

PART 2

Moisture Determination.—In order to get an idea of the progress of drying, final moisture content, and the effects of heating on the weight of the materials used in the granulation, moisture determination experiments were carried out in each case in the following manner: A representative sample of the powder mixture to be granulated subsequently was weighed in a previously tared weighing bottle and either subjected to heating in a heating oven at temperatures permissible by the nature of the materials involved for a period ranging from two to five hours to give constant weight, or dried in a vacuum desiccator over sulfuric acid for a period ranging from twenty-four to seventy-two hours to give a constant weight. This procedure was also carried out on a representative sample of the granulated material and a representative sample of the granular material at the end of each run under the lamps.

It was found that moisture of the granulation was cut down approximately by 10%, during the first run, in cases with actual moisture content below 20%. (The time for the first run was seventeen minutes.) In such cases a second run under a few or all the lamps brought the moisture content of the material to a level which permitted successful compression in almost all cases. But in the cases where the actual moisture content was above 20%, heating under the lamps for the same length of time did or did not dry the material so as to be satisfactorily compressed and in some instances a third and even a fourth run was necessary. Examples of such instances with the moisture content after the final run are as follows:

1. Antacid—11.6% after the fourth run.
2. Calcium Carbonate (A)—2.65% after the fourth run.
3. Calcium Carbonate (B)—0.69% after the third run.
4. Buchu and Atropine Compound—7.7% after the third run.

TABLE I.—GRANULATIONS OF INORGANIC COMPOUNDS

Distance: 15 inches
Thickness: 9/8 inch

No.	Name	Granule Size (Mesh)	Bulb (Watts)	Time in Min.	Temp. (Max.), °C.	Results of Drying	Lubricant	Compression
1	Antacid	10	375	63	46.5	Wet	No. 1	P
2	Bismuth Subgallate	10	500	30	63	Dried	No. 1	S
3	Bismuth Subnitrate	16	375	17	48	Dried	No. 1	S
	Bismuth Subnitrate	16	375	24	48	Dried	No. 1	S
4 A	Calcium Carbonate	16	500	50	54	Dried	5% T	B
	Calcium Carbonate	16	500	62	54	Dried	5% T + 5% ST	B
4 B	Calcium Carbonate	10	500	53	60	Dried	No. 1	S
5	Calcium Gluconate	10	500	35	54	Dried	6% T	S
6	Calcium Lactate	10	500	35	58	Dried	6% T	S
7	Calcium Phosphate	16	500	24	59.5	Dried	No. 1	S
8	Calcium Sulfide	10	500	17	61.5	Dried	4% T	S
9	Calomel	10	500	17	61.5	Dried	No. 1	S
10	Ferrous Sulfate	16	500	17	54	Dried	No. 1	S
11	Lithium Citrate	10	500	17	62	Dried	No. 1	S
	Lithium Citrate	10	500	24	62	Dried	No. 1	S
12	Magnesium Salicylate	10	500	21	51.5	Dried	No. 1	S
13	Magnesium Sulfate	10	375	15	...	Dried	2% T	S
14	Magnesium Trisilicate	10	500	35	54	Dried	No. 1	S
15	Mercurous Iodide, Yellow	16	250	24	44.5	Gray on top	6% T	S
16	Potassium Citrate	10	500	17	54	Dried	No. 1	P
17	Sodium Acid Phosphate I	16	375	17	50	Dried	5% T	STI
	Sodium Acid Phosphate II	16	500	14	53	Del.	...	...
	Sodium Acid Phosphate III	16	250	30	45	Dried	5% T + 5% ST	STI
18	Sodium Benzoate	10	500	17	55.5	Dried	No. 1	S
	Sodium Benzoate	10	500	24	55.5	Dried	No. 1	S
19	Sodium Chloride I	10	500	15	51	Dried	No. 1	C
							3% T	S
	Sodium Chloride II	16	375	17	53	Dried	No. 1	C
20	Sodium Citrate	16	500	17	58	Dried	No. 1	S
21	Sodium Salicylate	10	500	24	59.5	Dried	No. 1	S
22	Strontium Salicylate I	10	500	17	55	Dried	No. 1	C
	Strontium Salicylate II	10	500	14	51	Dried	No. 1	C
							6% T	LC

TABLE II.—GRANULATIONS OF ORGANIC COMPOUNDS

Distance: 15 inches
Thickness: 3/8 inch

No.	Name	Granule Size (Mesh)	Bulb (Watts)	Time in Min.	Temp. (Max.), °C.	Results of Drying	Lubricant	Compression
1	Aminopyrine I	10	500	30	49	Dried	No. 1	C
	Aminopyrine II	10	500	17	42	Wet	...	...
		..	375	18	51	Dried	No. 1	C
							6% T	LC
2	Caffeine, Citrated	10	500	17	62	Dried	No. 1	S
3	Calcium Pantothenate	10	500	17	51	Dried	5% T	S
4	Charcoal and Pepsin	16	500	20	...	Dried	No. 1	VS
5	Ephedrine Sulfate	10	500	17	62	Dried	1% MS + 1% ST	C
6	Nicotinamide	16	500	21	53	Dried	5% T	S
7	Nicotinic Acid	16	500	17	67	Dried	6% T	S
8	Phenacetin	10	500	24	48.5	Dried	No. 1	S
9	Phenobarbital	10	500	17	53	Dried	No. 1	C
							4% T	S
							4% ST	S
							4% MS	C
10	Riboflavin	16	500	17	55	Dried	5% T	S
11 A	Sulfadiazine	16	500	29	51	Dried	6% T	S
11 B	Sulfadiazine	16	500	29	56	Dried	6% T	S
12	Sulfamerazine	16	500	32	61	Dried	6% T + 3% ST	S
13 A	Sulfanilamide	16	500	17	51	Dried	No. 1	C
13 B	Sulfanilamide I	16	500	17	53	Dried	No. 1	C
	Sulfanilamide II	10	500	17	55.5	Dried	No. 1	C
14	Sulfapyridine	16	500	24	62	Dried	6% T	S
15	Sulfathiazole I	10	375	30	47	Dried	No. 1	S
	Sulfathiazole II	10	500	24	50	Dried	No. 1	S
16	Tannic Acid	10	500	5	...	Fusion	...	...
17	Thiamine Chloride	16	500	17	47	Dried	5% T	S
18	Vitamin C	16	500	24	56	Dried	6% T	S
19	Yeast	16	500	24	55	Dried	No. 1	VS

TABLE III.—COMPOUND GRANULATIONS

Distance: 15 inches
Thickness: 3/8 inch

No.	Name	Granule Size (Mesh)	Bulb (Watts)	Time in Min.	Temp. (Max.), °C.	Results of Drying	Lubricant	Compression
1	Acetanilid, Caffeine and Soda Compound	10	500	30	51	Dried	No. 1	S
2	Acetanilid and Hyoscyamus Compound	10	500	17	51	Dried	5% T	C
3	Aloin, Strychnine, and Belladonna Compound	16	500	24	61	Dried	10% T	S
4	Benzocaine Compound	16	500	33	60.5	Dried	2% MS + 3% ST	S
5	Brown Compound	16			Not Performed			
6	Buchu and Atropine Compound I	16	500	17	...	Damp	...	...
	Buchu and Atropine Compound II	16	250	35	...	Dried	7% T	B
7	Calomel, Rhubarb and Colocynth Compound	16	500	17	67	Dried	10%	S
8	Damiana, Nux Vomica, and Zinc Phosphide Compound	16	500	17	61	Dried	10% T	S
9	Extract Cascara Sagrada	16	500	17	63.5	Dried	10% T	S
10	Flatulence	16	500	17	72	Dried	10% T	S
	Flatulence	16	500	24	72	Dried	10% T	S
11A	Laxative I	10	500	17	70	Dried	10% T	S
	Laxative I	10	500	24	70	Dried	10% T	S
11 B	Laxative I	10	500	17	66.5	Dried	5% T	SO
12	Ox Bile, Pepsin, and Pancreatin	16	500	17	...	Dried	No. 1	S
13	Pancreatin and Pepsin	10	500	17	...	Dried	No. 1	S
14 A	Rhubarb and Ipecac Compound	10	500	29	57	Dried	5% T	S
14 B	Rhubarb and Ipecac Compound	16	500	24	57	Dried	5% T	S

PART 3

Assay of the Granulations.—In order to establish absence of any decomposition in the various granulations dried under the infrared lamps, representative samples of granulations were assayed. The nature of the ingredients contained therein suggested probabilities of chemical changes during the drying operation. Such changes, if any, however, could be attributed, in part, to conditions other than heating, to which the materials might have been required to be exposed during the entire process. The results of this part of the experimental are depicted in Table IV.

CONCLUSIONS

From the work done under the present project the following conclusions were drawn:

1. Heating by infrared lamps was rapid and heat distribution was uniform throughout.

2. Infrared heating is applicable to the drying of a variety of tablet granulations provided consideration is given to the nature of the ingredients involved.

3. The following tablet granulations do not lend themselves to successful drying under the infrared heat:

(*a*) Yellow Mercurous Iodide.
(*b*) Sodium Acid Phosphate.
(*c*) Tannic Acid.
(*d*) Dextrose.
(*e*) Buchu and Atropine Compound.

4. The period of drying and the maximum temperature attained during the drying operation depend on the moisture content, distance, thickness, capacity of the bulb, and the nature of the materials involved. (Drying period did not exceed twenty minutes in 50 per cent, thirty minutes in 36 per cent, and forty minutes in 8 per cent of the cases tried. It exceeded forty minutes in 4 per cent of the cases tried. The maximum temperature attained during the drying operation did not exceed 50° in 21 per cent, 60° in 75 per cent, and 70° in 98 per cent of the cases tried. It exceeded 70° in only 1 case.)

5. Granulations with moisture content below 15 per cent can be satisfactorily dried within twenty-five minutes by using lamps of maximum capacity permissible by the nature of the material.

6. No one particular capacity of the bulb will effect satisfactory drying in all cases without

TABLE IV.—ASSAY OF THE GRANULATIONS[a]

No.	Name	Official Requirements	% Labeled	% Found[b]	% of the Labeled Amount
1	Acetophenetidin	⩽94% & ⩾106% $C_{10}H_{13}O_2N$	92.653	90.252	97.408
2	Aminopyrine	⩽93% & ⩾107% $C_{13}H_{17}N_3O$	90.5	85.802	94.8
3	Ascorbic Acid	⩽95% & ⩾120% $C_6H_8O_6$	10.173	10.26	100.86
4	Caffeine, citrated	⩽45% & ⩾55% of the labeled amount of citrated caffeine	19.55	10.006	51.18
5	Calcium Sulfide U. S. P. IX	Crude Calcium Sulfide contains 55% CaS	19.482	11.41	58.56
6	Ephedrine Sulfate	⩽93% & ⩾107% $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$	10.345	10.734	103.76
7	Ferrous Sulfate	⩽95% & ⩾110% $FeSO_4 \cdot 7H_2O$	64.777	61.83	95.45
8	Magnesium Salicylate	.....	93.75	92.013	98.147
9	Mercurous Chloride, Mild	⩽92.5% & ⩾107.5% HgCl	19.607	21.643	110.38
10	Mercurous Iodide, Yellow	⩽91% & ⩾109% HgI	5.9535	6.226	104.41
11	Nicotinamide	⩽95% & ⩾115% $C_6H_6N_2O$	7.0	7.31	104.43
12	Nicotinic Acid	⩽95% & ⩾115% $C_6H_5O_2N$	9.124	8.64	94.695
13	Phenobarbital	⩽94% & ⩾106% $C_{12}H_{12}N_2O_3$	9.6385	9.669	100.31
14	Riboflavin	⩽95% & ⩾120% $C_{17}H_{20}N_4O_6$	0.3704	0.370	100
15 A	Sodium Bicarbonate (starch paste granulated)	⩽92.5% & ⩾107.5% $NaHCO_3$	95.238	102.238[c]	107.35
15 B	Sodium Bicarbonate (Alcohol granulated)	⩽92.5% & ⩾107.5% $NaHCO_3$	100	107.03[+]	107.03
16	Strontium Salicylate	.....	67.67	67.54	99.807
17	Sulfadiazine	⩽95% & ⩾105% $C_{10}H_{10}N_4O_2S$	58.05	57.594	99.21
18	Sulfamerazine	⩽95% & ⩾105% $C_{11}H_{12}N_4O_2S$	67.476	65.954	97.744
19	Sulfanilamide	⩽95% & ⩾105% $C_6H_8N_2O_2S$	64.53	62.93	97.52
20	Sulfapyridine	⩽95% & ⩾105% $C_{11}H_{11}N_3O_2S$	72.254	71.936	99.56
21	Sulfathiazole	⩽95% & ⩾105% $C_9H_9N_3O_2S_2$	94.308	95.14	100.9
22	Thiamine Hydrochloride	⩽95% & ⩾120% $C_{12}H_{17}ClN_4OS$—HCl	0.4576	0.455	99.43

[a] Assay procedures as given in the National Formulary or United States Pharmacopœia, as the case may be.
[b] Figures represent average of four assays. Figures for nicotinamide, nicotinic acid, riboflavin, and thiamine hydrochloride are based on results of assays carried out by the laboratories of Eli Lilly and Company, Indianapolis, Ind.
[c] In the case of sodium bicarbonate, the U. S. P. procedure includes any carbonate that might be present in the granulation. To determine the extent to which the bicarbonate has been changed into carbonate, an assay procedure given in "Scott's Standard Methods of Chemical Analysis" (5) was used. The amount of carbonate in the sodium bicarbonate granulations was found to be 6–13%.

unusually extending the drying cycle or raising temperatures beyond safe levels.

7. Thickness of 3/8 inch and distance of 15 inches seem to enable satisfactory drying. (No attempt was made to determine the optimum thickness and the optimum distance for each case.)

8. No deterioration in the nature of the chemicals that can directly be attributed to the effects of infrared heat could be established.

9. Infrared drying of tablet granulations can be successfully carried out on a large scale by suitably designing a belt conveyor or similar unit to handle the necessary volume of materials.

10. Great savings in floor space are effected by using infrared lamps as a source of heat. The experimental unit used in this project, having a capacity of 60 pounds, on an average, for an eight-hour working schedule, occupies a floor space of about 80 cubic feet while a unit using any other form of heating to do the same job in ordinary cases could be estimated to require twice as much space.

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Determination of the Stability of Epinephrine by Optical Rotation*

By HAROLD ROSENBLUM, ROBERT GOLDMAN and HERMAN FELDMAN

A method that can be adapted to dilute solutions and oil suspensions of epinephrine for the determination of stability is described. It is recommended that a decrease in optical rotation in liquid preparations of epinephrine preparations should be checked biologically to determine their true physiological activity.

INSTABILITY of epinephrine especially in solution as the hydrochloride has been a matter of interest and concern to all pharmaceutical houses who handle this item. Attempts have been made to overcome this instability with varying degrees of success by the use of stabilizers, usually antioxidants, such as ascorbic acid, amino acetic acid, sodium bisulfite, and also by employing the racemic form as a nonofficial preparation.

The picture is further complicated by the lack of a suitable, rapid, and convenient method for determining the potency of this medicinal. The bioassay is an expensive procedure which requires the services of someone specially trained in the technique of this method and therefore is excluded as a routine method of analysis by the analytical chemist. The colorimetric methods on

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the other hand do not differentiate between the laevo and dextro forms and may show 100% potency when compared with U. S. P. epinephrine, whereas the physiological potency may be slightly higher than 50% if the epinephrine is racemic or has racemized on standing.

In view of the above difficulties this study was undertaken. The method of analysis was based upon the specific rotation of epinephrine when dissolved in water containing a slight excess of hydrochloric acid. Official preparations are epinephrine, epinephrine inhalation (1:100) and epinephrine injection (1:1000). The monograph for the powder fixes the specific rotation in half-normal hydrochloric acid at not less than $-50°$ and not more than $-53.5°$. No rotation is given for the two solutions.

Upon analyzing the results obtained by determining the specific rotation of about 20 lots of epinephrine powder by dissolving the sample in a slight excess (2–5%) of hydrochloric acid, it was found that in a large majority of samples the rotation was between $-51.5°$ and $-52.5°$, the average of all lots being $-52°$. This value was then taken as the theoretical upon which to base the per cent epinephrine in solution. This value furthermore is, for practical purposes, the midpoint in the U. S. P. tolerances.

The difficulties in determining the specific rotation of a 1:1000 solution of epinephrine or a suspension of epinephrine in oil are self-evident.

The observed rotation for a 1:100 solution, using a 200-mm. tube, would be about $-1°$ and for a 1:1000 solution $-0.1°$. Due to the small reading for the 1:1000 solution, it was found advisable to vacuum concentrate the solution to a 1:100 or 1:200 concentration before determining the rotation. Trial determinations on a 1:1000 solution prepared by diluting a 1:100 solution of predetermined specific rotation with normal saline solution containing 0.1% sodium bisulfite proved successful. The specific rotation of a 1:100 solution was found to be $-52°$, and after dilution and vacuum concentration $-53°$. Similar values were found on repeated tests.

For the determination of the epinephrine suspended in oil the procedure had to be modified to remove the epinephrine from the oil before determining the optical rotation. This was accomplished by extracting the epinephrine with 0.1 *N* hydrochloric acid. Values for several samples tested by this method are listed in Table I.

Table I.—Comparative Values Obtained by Biological Assay and Specific Rotation

Sample	—Epinephrine by— Biological Assay, %	Specific Rotation,[a] %
Epinephrine injection 1:1000, No. 143	95.7	100.4
Epinephrine injection 1:1000, No. 272	99.4	104
Epinephrine injection 1:1000, No. 045	100	101
Epinephrine in oil 1:500, No. 096	100	97
Epinephrine in oil 1:500, No. 285	100	103

[a] Limit of error ±2.2%.

EXPERIMENTAL

The U. S. P. dog assay was used for the biological determination.

The method given in "New and Non-Official Remedies 1946," page 297, was used for the colorimetric determination.

Determination of Epinephrine Injection 1:1000 by Optical Rotation.—Place 125 cc. of the epinephrine solution 1:1000, accurately measured, in a 250-cc. standard taper flask and set up the apparatus for vacuum concentration as shown in Fig. 1. The flask contains a capillary tube which dips below the surface of the liquid to be concentrated. Do not use a rubber or cork stopper for the flask as this will cause the solution to become discolored during the concentration.

Concentrate under a vacuum of not more than 15 mm. mercury. Do not allow the water bath to rise above 50°. The vacuum concentration is accomplished as rapidly as possible and the solution concentrated to about 15 cc. Allow the flask to attain atmospheric pressure by opening the capillary tube. Transfer the solution quantitatively to a 25-cc. volumetric flask with the aid of several centimeters of water containing 0.1% sodium bisulfite, adjust the temperature to 25°, and then dilute the solution to 25 cc. with distilled water. Observe the optical rotation in a 200-mm. tube at 25°.

$$\% \text{ of Labeled Strength} = \frac{\alpha \times 10{,}000}{2 \times 52 \times X \times C}$$

α = observed rotation
X = labeled strength in per cent
C = the number of times the solution has been concentrated

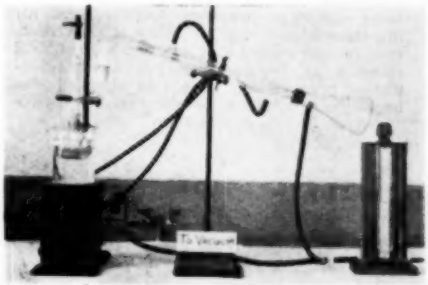

Fig. 1.—Apparatus for vacuum concentration of epinephrine solutions.

Determination of Epinephrine in Oil by Optical Rotation.—Shake the ampuls or vials containing the preparation to disperse thoroughly the suspended epinephrine, and then remove the material with the aid of a syringe and needle. Collect the material in a 125-cc. glass stoppered flask, shake well, and withdraw an aliquot sufficient to furnish 100 mg. of epinephrine and transfer to a dry separatory funnel. Add exactly 20 cc. of 0.1 *N* hydrochloric acid and shake the mixture well for five minutes. Allow to stand until the layers separate, draw off the lower aqueous layer and filter through a dry filter into a dry container. Transfer the filtrate to a 200-mm. polariscope tube and determine the angular rotation of the solution at 25°.

The calculations are the same as for the 1:1000 aqueous solution.

DISCUSSION

Solutions of epinephrine hydrochloride (1:100) of several manufacturers were assayed biologically, colorimetrically and by their specific rotation. The results are recorded in Table II. With deteriorated solutions the colorimetric method gave the highest results, the biological method the next highest, the optical rotation the lowest results. This can be attributed to racemization of the *l* form to the *d* form. For example, Lot No. 092, when freshly prepared, showed approximately 100% epinephrine by all three methods. Upon standing several months, the solution apparently partially racemized as indicated by the fact that the colorimetric method still showed 100% of potency, the bioassay only 91% and the optical rotation 70%.

This lot shows by optical rotation that 15% of the laevo form still predominates. The colorimetric method still shows 93% epinephrine. It appears therefore that 39% has been converted to the dextro form, yielding 78% of racemic epinephrine. Therefore, 39%, which is the laevo fraction of the racemic epinephrine plus 15% laevo equals 54%. The biological method should show about 54% activity. Actually 55% was found which can be considered within the experimental error.

A comparison of the biological assay with the average of the physical measurements for each sample (see Table II) shows a fairly close agreement in several instances. The accuracy of each determination is subject to an error of approximately ±3%. This error may be cumulative, and the actual variation between biological and physical measurements can be about ±10%.

However, these results indicate that by averaging the values obtained by the colorimetric method and the specific rotation (Table II), the biological activity can be estimated. The concentration of an unknown solution by optical rotation using a 200-mm. tube can be calculated from the formula:

$$\% \text{ Epinephrine} = \frac{\alpha \times 100}{2 \times C \times 52}$$

C = number of times solution has been concentrated. For example: 100 cc. concentrated to 20 cc. = 5 times.

This value averaged with the colorimetric determination should approximate the biological activity.

TABLE II.—EPHINEPHRINE DETERMINATION

Sample	Epinephrine By: Colorimetric Assay, %	Specific Rotation, %	Biological Assay, %	Average of Specific Rotation and Colorimetric Assay, %
Epinephrine, inhalation 1:100, No. 190, prepared on 7-3-47	115	105	97	110
Epinephrine, inhalation 1:100, No. 190, retested on 4-29-48	115	70	100	92.5
Epinephrine, inhalation 1:100, No. 092, prepared on 4-1-47	102	100	96.4	101
Epinephrine, inhalation 1:100, No. 092, retested on 4-29-48	100	70	91	85
Epinephrine, inhalation 1:100, C2029	102.5	15	55	59
Epinephrine, inhalation 1:100, 15928	93	15	55	54

The difference in the results can be postulated as follows: upon racemizing, one molecule of epinephrine is converted to the dextro isomer which neutralizes the rotation of another laevo molecule. Therefore, if half the epinephrine racemized to the dextro form, there would be no rotation and the solution would have slightly more than 50% of its potency (due to the slight activity of the *d* form) by biological assay. The solution would still show 100% of potency by colorimetric assay due to the fact that this method cannot distinguish between the dextro and laevo forms. The results of Lot No. 15928 as shown in Table II will best illustrate this point.

The above results show that racemization or deterioration is readily detected by checking the optical rotation periodically. Furthermore, it is our experience that if racemization occurs in solution, the most rapid fall in rotation will occur within a month after the solution is prepared. The rotation drops to about −35°, after which further racemization does not occur at all or will progress at a very much slower rate. The cause for the racemization has not been definitely placed and is under further investigation.

The widespread belief that the loss in physiological potency invariably is indicated by the production of a brown color and precipitate is

erroneous. The addition of bisulfite does not prevent racemization or decomposition but may prevent discoloration from decomposition products. A solution of epinephrine hydrochloride containing bisulfite may lose a large part of its physiological properties and yet it may remain colorless or turn only slightly yellowish. The limitation on this method for determining the potency by optical rotation of the solution is of course the brown color which may be produced by oxidation in some solutions. However, this color in itself would automatically eliminate the material from sale or from being used by the patient. For the purpose of controlling the product and determining the shelf life, the optical rotation method has been found to be a simple, rapid, and inexpensive procedure.

SUMMARY

1. Solutions of epinephrine hydrochloride can be constantly checked for stability by determining the optical rotation.

2. Deterioration can be detected most readily by this means since the effect is most pronounced upon the rotation.

3. Evidence is presented that deterioration can be caused by racemization.

4. The method can be adapted to solutions of 1:1000 and also to oil suspensions.

5. A solution showing a drop in optical rotation should be checked biologically to determine its true physiological potency.

The Bactericidal Action of Oxygen-Liberating Substances on Oral Microorganisms. II. Studies with Oral Spirochetes*

By WILLIAM A. NOLTE,† MARGUERITE A. EDWARDS‡ and THOMAS C. GRUBB

Two strains of oral spirochetes, isolated from cases of Vincent's infection, were cultured in a thioglycollate-blood-infusion medium and inoculated into various concentrations of sodium perborate, hydrogen peroxide, and a neutralized perborate dentifrice. Subcultures made after thirty seconds, one and two minutes' contact were incubated at 37° and examined by the dark-field method after seven and fourteen days' incubation. Controls included in each experiment warranted the conclusion that the absence of growth in the subculture tubes resulted from the germicidal action of the oxygen-liberating substances *per se*. The perborate dentifrice diluted 1:10 with water, saliva or saliva plus 10% serum killed billions of spirochetes in less than thirty seconds, in most instances. The perborate dentifrice, hydrogen peroxide and sodium perborate diluted with water exhibited approximately equal spirochetacidal action when compared on the basis of equal oxygen content. Although all three products were equally effective in the lower dilutions usually employed for oral use, the strong alkalinity of sodium perborate and the high acidity of commercial hydrogen peroxide solutions are considered undesirable properties of products intended for oral application.

RECENTLY a paper (1) from this laboratory appeared which described the rapid germicidal action of sodium perborate, hydrogen peroxide and a neutralized sodium perborate dentifrice on fusiform bacilli suspended in water, saliva, or saliva plus serum. In order to complete the study of the action of oxygen-liberating substances on oral microorganisms, especially those associated with Vincent's infection, experiments with these substances were carried out using two strains of oral spirochetes as the test organisms.

EXPERIMENTAL

Two strains of oral spirochetes (Nos. 69 and 78) isolated from cases of Vincent's infection[1] were cultivated in the following medium:

Sodium thioglycollate, Gm.	1
Dextrose, Gm.	10
Agar, Gm.	0.5
Beef infusion with 1.0% thiopeptone, cc.	1000

The medium was adjusted to *p*H 7.8, tubed in 10 cc. amounts and autoclaved twenty minutes at 15 lb. Just before use, 0.5 cc. of citrated rabbit's blood was added to each tube. The stock cultures were continuously maintained by inoculating 0.5

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[1] The writers are indebted to Dr. Ruth Wichelhausen for the cultures used in this study.

cc. of a ten-day-old culture into fresh tubes which were in turn incubated ten days at 37° before transferring to fresh media.

Since it was believed important to know the number of organisms employed in the germicidal tests, a method was devised, similar to the Breed count in principle, which was used to count the number of spirochetes per dark field and translated into the number of organisms per cubic centimeter. When checked by growth of the organisms in serial dilutions, the dark field counts showed close agreement.

In the first series of experiments the germicidal action of a neutralized sodium perborate dentifrice[2] was determined as follows:

Five-tenths of a cubic centimeter of a ten- to twelve-day-old culture of spirochetes was added to 5 cc. of the neutralized perborate dentifrice diluted 1:5 in distilled water, undiluted sterile saliva or undiluted sterile saliva plus 10% rabbit serum. After a contact interval of thirty seconds, one and two minutes at room temperature, a 4-mm. loopful of the mixture was transferred to tubes of the spirochete medium which were incubated at 37° and examined for growth by the dark field method after seven and fourteen days' incubation.

Two controls were always included in each experiment reported in this paper. The first was a "carry-over" control which consisted of inoculating a loopful of the most concentrated dilution of the dentifrice, sodium perborate or hydrogen peroxide used in each test, into the subculture medium, followed by a loopful of the test organisms. Since these controls always showed growth it indicated that the amount of oxygen-liberating substance carried over by the loop did not produce bacteriostasis in the subculture medium and hence any tubes failing to show growth had been inoculated with organisms killed by the test substances. The second type of control included in all experiments consisted of the inoculation of 0.5 cc. of the test organism into water, undiluted saliva or undiluted saliva plus 10% serum and allowed to remain in contact with these substances for the longest period of medication contact employed in each experiment (i.e., two minutes), before a loopful was transferred to the subculture medium. Since these "menstruum" controls always showed growth in the subculture tubes it was concluded that contact of the spirochetes with water, saliva or saliva plus serum *per se* did not contribute significantly to the killing of the test organisms. Since these two types of controls showed satisfactory results in each of the experiments reported, it is believed that the failure of growth to appear after contact with the various oxygen-liberating agents represents the results of a true germicidal test.

In the first series of experiments a 1:5 dilution of the dentifrice was employed for testing because it has been estimated that this is the approximate concentration of a powdered dentifrice in the mouth under the usual conditions of use. The results of this experiment indicated in Table I show that both spirochete strains were killed after not more than thirty seconds' contact with the neutralized perborate dentifrice diluted with water, saliva, or saliva plus serum. Since the inoculum in these experiments contained approximately 18,580,000,000 spirochetes (the majority of which appeared to be motile), it is evident that the dentifrice was markedly germicidal for these organisms.

Since the perborate dentifrice proved so effective in killing spirochetes in low dilutions, a study was made to determine its activity in higher dilutions. The methods employed were the same as those described for the preceding experiments except that dilutions of the dentifrice from 1:10 to 1:80 were made in sterile water, saliva, or saliva plus 10% serum. The inocula contained approximately 20,000,000,000 spirochetes. The results of this test are shown in Table II.

TABLE I.—SPIROCHETACIDAL ACTION OF THE NEUTRALIZED PERBORATE DENTRIFRICE DILUTED 1:5 ON ORAL SPIROCHETES

Contact Time	Strain No. 69			Strain No. 78		
	Water Dil.	Saliva Dil.	Saliva + Serum Dil.	Water Dil.	Saliva Dil.	Saliva + Serum Dil.
30 sec.	−[a]	−	−	−	−	−
1 min.	−	−	−	−	−	−
2 min.	−	−	−	−	−	−
Control	+[b]	+	+	+	+	+

[a] − = No growth in subculture tubes.
[b] + = Growth in subculture tubes.

The action of the dentifrice on spirochetes shows the usual time-dilution relationship found with bacteria subjected to germicides; i.e., the higher the dilution the greater the length of time required to kill the organisms. In the case of strain No. 69 the presence of serum in the saliva caused some reduction in the activity of the dentifrice. Strain No. 78 showed some inconsistencies which are difficult to interpret, since the presence of saliva alone had a greater inhibitory action on the dentifrice than saliva plus serum. In general the dentifrice killed the spirochetes within one minute when diluted 1:20.

Since sodium perborate or hydrogen peroxide is frequently used in dental practice to treat oral infections it was considered of interest to compare their *in vitro* bactericidal action on oral spirochetes with that of the neutralized perborate dentifrice. The test methods were the same as those used in

[2] The dentifrice (Neutrox) has the following composition: sodium perborate monohydrate (U. S. P. dehydrated), 30%; monocalcium phosphate, 30%; tricalcium phosphate, 23.87%; magnesium carbonate, 10%; saccharin soluble, 0.3%; sodium chloride, 2%; menthol 0.33%; oil of peppermint, 0.5%; methyl salicylate, 0.65%; oil of anise, 0.042%; liquid petrolatum light, 1.81%; and sodium lauryl sulfoacetate, 0.5%. (U. S. Patent No. 2,094,671.)

TABLE II.—SPIROCHETACIDAL ACTION OF THE NEUTRALIZED PERBORATE DENTIFRICE DILUTED 1:10 TO 1:80 ON ORAL SPIROCHETES

Contact Time	Strain No. 69				Strain No. 78			
	1:10	1:20	1:40	1:80	1:10	1:20	1:40	1:80
Water Dilutions								
30 sec.	−[a]	+[b]	+	+	−	−	−	+
1 min.	−	−	+	+	−	−	−	+
2 min.	−	−	+	+	−	−	−	−
Control	+				+			
Saliva Dilutions								
30 sec.	−	+	+	+	+	+	+	+
1 min.	−	−	+	+	−	+	+	+
2 min.	−	−	+	+	−	−	+	+
Control	+				+			
Saliva plus Serum Dilutions								
30 sec.	+	+	+	+	−	−	+	+
1 min.	−	+	+	+	−	−	−	+
2 min.	−	+	+	+	−	−	−	−
Control	+				+			

[a] − = No growth in subculture tubes.
[b] + = Growth in subculture tubes.

the preceding experiments. In preparing the dilutions of the substances to be tested, the concentration of available oxygen per Gm. or cc. was first determined and then dilutions in sterile water were made so that the corresponding dilution of each substance contained the same amount of available oxygen; e.g., a 1:50 dilution of sodium perborate contained the same amount of available oxygen as a 1:20 dilution of the perborate dentifrice. In these experiments the strain No. 69 inoculum contained approximately 19,509,000,000 spirochetes and strain No. 78 contained 25,083,000,000 spirochetes. (See Table III.)

The comparison of the three oxygen-liberating substances on oral spirochetes shown in Table III indicates that most have approximately the same spirochetacidal action. This suggests that the principal agent in each substance is the hydrogen peroxide or nascent oxygen liberated in solution.

DISCUSSION

The results of this study indicate that oxygen-liberating substances have a rapid spirochetacidal action *in vitro;* even in the presence of saliva and serum included to simulate *in vivo* conditions of use. A direct comparison of the relative effectiveness of these substances on fusiform bacilli previously reported (1) and on oral spirochetes is not possible because inocula containing approximately a hundred times greater number of organisms were used in the spirochete experiments. However, with this factor taken into consideration the results suggest that the oxygenating substances tested kill spirochetes more rapidly than they do fusiform bacilli. Since it has been demonstrated in the two papers of this series that oxygen-liberating substances are actively germicidal *in vitro* against both types of organisms associated with Vincent's

TABLE III.—COMPARISON OF SPIROCHETACIDAL ACTION OF THE NEUTRALIZED PERBORATE DENTIFRICE WITH SODIUM PERBORATE AND HYDROGEN PEROXIDE

Contact Time	Strain No. 69					Strain No. 78				
	1:10	1:20	1:40	1:80	1:160	1:10	1:20	1:40	1:80	1:160
Perborate Dentifrice										
30 sec.	−[a]	+[b]	+	+	+	−	−	+	+	+
1 min.	−	−	+	+	+	−	−	+	+	+
2 min.	−	−	−	−	+	−	−	+	+	+
Control	+					+				
Hydrogen Peroxide[c]										
	1:4	1:8	1:16	1:32		1:4	1:8	1:16	1:32	
30 sec.	+	+	+	+		+	+	+	+	
1 min.	−	−	+	+		−	+	+	+	
2 min.	−	−	−	−		−	−	−	−	
Control	+					+				
Sodium Perborate[d]										
		1:50	1:100	1:200	1:400		1:50	1:100	1:200	1:400
30 sec.		+	+	+	+		+	+	+	+
1 min.		−	+	+	+		+	+	+	+
2 min.		−	−	−	−		−	−	+	+
Control		+					+			

[a] − = No growth in subculture tubes.
[b] + = Growth in subculture tubes.
[c] Hydrogen peroxide, U. S. P.
[d] Sodium perborate monohydrate, U. S. P.

infection, it is believed that these experiments justify the widespread reliance placed in these agents for the treatment of this infection.

The choice between the three oxygenating substances for oral usage, briefly discussed in the previous report (1), may be considered here. While sodium perborate, hydrogen peroxide and the neutralized perborate dentifrice all appear to be equally effective against oral spirochetes and fusiform bacilli in the low dilutions commonly employed in the mouth, the fact that sodium perborate is strongly alkaline (*p*H 9.9) and commercial hydrogen peroxide is quite acidic (*p*H 3.7–4.9), suggests that these two agents are not so desirable for oral application as one which has been carefully buffered to an approximately neutral state (2) like the neutralized perborate dentifrice employed in this study.

SUMMARY

Carefully controlled *in vitro* tests with sodium perborate, hydrogen peroxide and a neutralized perborate dentifrice indicated that these substances were rapidly spirochetacidal for two strains of oral spirochetes. The perborate dentifrice diluted 1:10 with water, saliva, or saliva plus 10 per cent serum killed approximately 20,000,000,000 spirochetes in less than one minute. Although the oxygen-liberating substances included in this study appear to have approximately equal spirochetacidal activity when compared in dilutions of equal oxygen content, the perborate dentifrice would appear to be the most suitable for oral application because of its neutral reaction.

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The Determination of Thymol and Carvacrol in Spanish Thyme Oils*

By EDWARD E. LANGENAU

The definition of thyme oil in The National Formulary, Eighth Edition, excludes those oils which contain carvacrol as the main phenolic constituent. However, in the monograph no analytical procedure is incorporated to distinguish between the carvacrol type and the thymol types of oils. This paper was written in order to initiate the accumulation of data which will lead to a satisfactory test. A procedure is given for the separation of phenols from thyme oils; the behavior of known mixtures of such separated phenols at fixed temperatures, when seeded with a crystal of thymol, is described.

ACCORDING to Guenther (1, 2), the commercial production of thyme oils in Spain results in two main types of oils:

1. Red thyme oils distilled from *Thymus vulgaris*, L. and *Thymus zygis*, L.; the phenolic constituents of this type of oil consist mainly of thymol, and contain only a small percentage of carvacrol. This oil is distilled in the provinces of Murcia and Almeria, an area entirely separated from the producing sections of Spanish origanum oil.

2. Origanum oils distilled from *Thymus capitatus*, Lk., now considered a separate genus, *Coridothymus capitatus* Rchb.; the phenolic constituents of this type consist mainly of carvacrol, and contain only a small percentage of thymol. This plant grows in the southern part of Spain (Andalusia), particularly in the provinces of Malaga, Cadiz, Huleva, Sevilla, and on the borders of Cordoba.

Oils containing thymol as the principal phenolic constituent are preferred generally in the trade to oils containing carvacrol. This preference may well be due to the fact that thyme oils were frequently used to replace the Indian ajowan seed oils as a source for crystalline thymol. In the United States, the manufacture of synthetic thymol on a large scale has resulted in a product that meets all U. S. P. requirements at a lower cost than natural thymol isolated from thyme oils.

For use in flavoring, the thyme oils are usually preferred because of their somewhat finer flavor character. An important quantitative difference in the phenol contents exists between the two types: Thyme oils usually contain about 50% phenols; origanum oils, about 65 per cent to 70 per cent phenols.

In the course of the following experiments, thyme oils were reconstituted from the separated

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nonphenolic portions of Spanish oils and from phenolic mixtures of thymol and purified carvacrol. In odor, little difference could be detected as the thymol-carvacrol ratio was altered. In view of these findings, both oils should be entirely satisfactory for imparting a pleasant phenolic odor to soaps; this is the most important commercial use of thyme and origanum oils today. For this purpose, the origanum oils offer the advantages of lower cost and greater strength.

For use in pharmaceuticals and medicinals, there is still some question as to the relative merits of the two types of oils. Much conflicting literature exists. It appears, however, that the medicinal value of the oils depends upon the presence of the phenols, thymol and carvacrol, and that both phenols have medicinal value. Livingston (3) made a careful comparison of thymol and carvacrol and found that toxicity and relative anthelmintic value of the two phenols were practically identical. Martindale (4) concluded that carvacrol is almost as actively germicidal as its isomer, thymol.

The National Formulary, Eighth Edition (5), defines thyme-oil as the "volatile oil distilled from the flowering plant of *Thymus vulgaris* Linné or *Thymus zygis* Linné and its variety *gracilis* Bois (Fam. *Labiatae*)." This definition excludes the carvacrol type of oil.

The following experiments were undertaken in an attempt to develop a practical laboratory method which would determine the percentage of thymol in the phenolic portion of the oil, and thus distinguish between the thymol and carvacrol types of oils.

Mayor (6) has reviewed several methods for the determination of total phenols in thyme oils and has described differences in the behavior of carvacrol and thymol. In the review, Mayor has described briefly several methods for the estimation of thymol in the presence of carvacrol; most of these are of value for the commercial separation of the two phenols, but leave much to be desired for a routine analytical procedure. The method of Sage and Dalton (7) appears the most promising; these authors determined congealing points for thymol-carvacrol mixtures and for mixtures of these phenols recovered from thyme oils of known composition. Their findings, however, are at variance with the data set forth in this paper.

EXPERIMENTAL

Part A—Phenols

Thymol.—The thymol used in the following experiments was of U. S. P. quality. The melting point was 49.5°–50.5° when determined in a capillary tube according to the procedure outlined in the U. S. P. XIII for testing materials of Class I (8). It showed a congealing point of +49.4° when supercooled to +40° and tested as described below under "Procedure." The thymol was considered to be of sufficient purity for use without further purification.

Carvacrol.—A commercial grade of carvacrol, warranted free from thymol by the manufacturer, was fractionated *in vacuo* with an 8-inch Vigreau column. The fraction +119°–121° at 15 mm. was collected; this represented 75% of the original product. The analysis of the fraction follows: $d_{25^\circ}^{25^\circ} = 0.977$; $\alpha_D^{25^\circ} = \pm 0°$; $n_D^{25^\circ} = 1.5236$; solubility 20°—clearly soluble in 2 volumes and more of 70% alcohol; solubility in alkali—clearly soluble in 10 volumes of KOH T.S.; upon standing, the solution developed a faint opalescence.

These data are in good agreement with the values reported in the literature for pure carvacrol (9).

To assure the absence of thymol, the following tests were carried out:

1. The carvacrol was supercooled to −20° and seeded with a small crystal of thymol: no growth of thymol crystals was observed after four hours at this temperature.

2. A mixture of 9.500 Gm. of thymol and 0.500 Gm. of carvacrol showed a congealing point of +46.2° when supercooled to +40° and tested as described below under "Procedure"; a second mixture of 9.500 Gm. of thymol and 0.500 Gm. of eugenol of U. S. P. quality showed the identical congealing point, +46.2°, when treated in a similar manner. If the carvacrol had contained thymol, a difference in congealing points would have been expected.

3. The carvacrol was tested for the presence of thymol by the procedure of Klingstedt and Sundström (10). The phenol was converted to the methyl ether by means of dimethyl sulfate. This ether was saturated with gaseous hydrogen chloride and a solution of sodium nitrite was added; no crystalline nitroso compound was formed. According to Klingstedt and Sundström, if thymol is present, a crystalline nitroso thymol will result.

Mixtures of Thymol and Carvacrol.—Mixtures of thymol and carvacrol were prepared from the phenols described above; the percentage of thymol was varied from 0% to 100%, by weight, in 10% steps. The analyses of the eleven samples thus prepared are given in Table I.

TABLE I.—ANALYSES OF MIXTURES OF PHENOLS

No.	Composition, % Thymol	Composition, % Carvacrol	$d_{25^\circ}^{25^\circ}$	$n_D^{25^\circ}$
0	0	100	0.977	1.5236
1	10	90	0.977	1.5235
2	20	80	0.976	1.5233
3	30	70	0.976	1.5232
4	40	60	0.975	1.5231
5	50	50	0.975	1.5230
6[a]	60	40	0.975	1.5230
7[a]	70	30	0.974	[b]
8[a]	80	20	[b]	[b]
9[a]	90	10	[b]	[b]
10[a]	100	0	[b]	[b]

[a] At a temperature of 25°, No. 6 through No. 10 showed a spontaneous separation of crystals within twenty-four hours. (No. 0 through No. 5 showed no such crystallization.)

[b] Mixture crystallized spontaneously during determination.

The differences in specific gravity and refractive index were insufficient for establishing the percentages of the phenols in the mixtures. The behavior of the mixtures at various temperatures was therefore investigated.

The use of the true congealing point did not prove entirely satisfactory. The congealing point was found to be dependent upon the degree of supercooling; with high percentages of thymol the degree of supercooling had little effect, but as the percentage of thymol was decreased, the effect of supercooling became very noticeable. Furthermore, at low temperatures and high percentages of carvacrol, the mixtures became very viscid and the crystals of thymol formed so slowly that no appreciable rise in temperature was observed in some cases.

The use of the end point of the melting range (the point at which the last trace of crystalline material disappears) also proved unsatisfactory. Overheating of the mixtures occurred frequently because of the high viscosity; temperature equilibrium was attained very slowly. Furthermore, the size of the crystals present, which is dependent upon the speed of crystallization, also affected the titer; large crystals dissolved slowly, so that the temperature of the mixture could be raised many degrees above the true melting point.

If the mixtures were maintained at a fixed temperature and a crystal of thymol added as a seed, the mixture would then crystallize if it were in a supercooled state. Since the mixtures could readily be supercooled many degrees below the true melting point, the introduction of the seed of thymol was essential to obtain reproducible results. Upon this behavior were the mixtures of phenols classified.

The procedure described below was used to study the behavior of the phenolic mixtures at various fixed temperatures. (See Table II.)

Procedure.—Into a test tube, 20 mm. in diameter and 100 mm. in length, introduce about 10 Gm. of the phenols. Insert a thermometer graduated in 0.1°. Warm gently until the contents are completely melted and a homogeneous liquid is obtained, free from any crystalline material. Insert the tube through a bored cork into a larger test tube, 40 mm. in diameter and 200 mm. in length, which is to act as an air jacket. Immerse the larger test tube to a depth of 170 mm. in a water bath maintained at a fixed temperature. Allow the contents of the inner tube to cool slowly without agitation until the temperature of the water bath is attained. Then introduce a small crystal of thymol to act as a seed and stir the contents vigorously with the thermometer, rubbing the latter on the sides of the tube with an up-and-down motion in order to induce rapid crystallization. Continue the stirring and rubbing as long as the temperature rises; record the highest point as the congealing point. If crystallization does not occur immediately, continue the stirring for five minutes and observe the condition of phenols to ascertain whether or not there is any haze or turbidity, resulting from the presence of small crystals throughout the liquid.

Part B—Thyme Oils

To study the behavior of thyme oils containing known percentages of thymol and carvacrol, such oils were prepared from the nonphenolic portion of a Spanish thyme oil and the thymol and carvacrol described in Part A.

Nonphenolic Portion of Thyme Oil.—A Spanish thyme oil of direct importation was used. The oil met all N. F. VIII requirements and had the following properties: $d_{25.0°}^{25.0°} = 0.9245$; $\alpha_D^{25°} =$

TABLE II.—BEHAVIOR OF MIXTURES OF PHENOLS

No.	Composition, % Thymol	Composition, % Carvacrol	Mixture Cooled to 0°	5°	10°	15°	20°	25°	30°	35°	40°	45°	50°
0	0	100	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
1	10	90	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
2	20	80	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
3	30	70	C.S. Notes 1 & 2	C.S. Notes 1 & 3	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
4	40	60	C.S. Note 4	C.I. Note 4	C.S. Note 1	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
5	50	50	Note 5	C.I. C.P. = 15.5°	C.I. C.P. = 18.4°	C.I. C.P. = 20.5°	C.S. Note 1	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
6	60	40	Note 5	C.I. C.P. = 17.0°	C.I. C.P. = 20.0°	C.I. C.P. = 21.6°	C.I. C.P. = 24.1°	C.S. Note 1	N.C.	N.C.	N.C.	N.C.	N.C.
7	70	30	Note 5	Note 5	Note 5	Note 5	C.I. C.P. = 29.0°	C.I. C.P. = 31.4°	C.I. C.P. = 33.1°	C.S. Note 1	N.C.	N.C.	N.C.
8	80	20	Note 5	Note 5	Note 5	Note 5	C.I. C.P. = 33.5°	C.I. C.P. = 35.4°	C.I. C.P. = 37.4°	C.I. C.P. = 39.0°	C.S. Note 1	N.C.	N.C.
9	90	10	Note 5	Note 5	Note 5	Note 5	C.I. C.P. = 40.0°	C.I. C.P. = 41.5°	C.I. C.P. = 42.3°	C.I. C.P. = 43.4°	C.I. C.P. = 43.4°	N.C.	N.C.
10	100	0	Note 5	Note 5	Note 5	Note 5	Note 5	Note 5	C.I. C.P. = 49.0°	C.I. C.P. = 49.2°	C.I. C.P. = 49.4°	C.I. C.P. = 49.4°	N.C.

Key: N.C. = no crystallization observed; C.I. = crystallizes immediately; C.S. = crystallizes slowly; C.P. = congealing point.

NOTE 1—No appreciable temperature rise observed.
NOTE 2—Mixture becomes turbid owing to presence of thymol crystals.
NOTE 3—Mixture becomes hazy owing to presence of thymol crystals.
NOTE 4—Temperature rise is slow; congealing point is not sharp and is not easily reproducible.
NOTE 5—Attempts to supercool to this temperature were unsuccessful; mixtures crystallized spontaneously.

$-1°56'$; solubility 25°—clearly soluble in 1 to 1.5 volumes of 80% alcohol and more; $n_D^{20°} = 1.4989$; phenol content = 48.5%; color—red.

Two and a half liters of the oil were stirred and shaken thoroughly with 12.5 L. of potassium hydroxide T.S. The mixture was permitted to separate overnight and the oily layer (1325 cc.) siphoned off. After two additional washings with 5 volumes (6,625 cc.) of potassium hydroxide T.S., the separated oily layer was shaken with 200 cc. of water and then with two 200-cc. portions of a saturated sodium chloride solution. The separated oil was dried with anhydrous sodium sulfate and filtered. The analysis of this nonphenolic portion of thyme oil is: $d_{25°}^{25°} = 0.878$; $\alpha_D^{25°} = -2°44'$; $n_D^{20°} = 1.4792$; solubility 25°—clearly soluble in 0 to 0.5 volumes 90% alcohol and more, hazy in 10 volumes of 80% alcohol; apparent phenol content = 1.0%.

The apparent phenol content probably is due to the solubility in water of some constituent other than a phenol, since upon separation of the aqueous potassium hydroxide layer (obtained from the assay) and subsequent acidification, no oily separation was observed. Extraction of the acidified aqueous layer with ether, and subsequent drying and removal of the ether gave only a trace of an oily liquid with a camphoraceous odor. This oily liquid did not have the characteristic phenolic odor of carvacrol or thymol; it gave no color reaction with a solution of ferric chloride (1:1000).

Reconstituted Thyme Oils.—Thyme oils were reconstituted by combining 50.0 Gm. of this nonphenolic portion and 50.0 Gm. of the mixtures of pure phenols containing 0% to 100% thymol. The analyses of the eleven samples of reconstituted oils is given in Table III.

Separation of Phenols.—The phenols were separated from these reconstituted thyme oils by the following method: Introduce 25 cc. of the oil into a 500-cc. separatory funnel. Add 250 cc. of potassium hydroxide T.S. Shake the mixture thoroughly for five minutes and then permit the two layers to separate overnight. Draw off the lower aqueous layer and filter into a 1000-cc. separatory funnel through a fluted filter paper previously wetted with water.[1] Add 100 cc. of dilute sulfuric acid (1:3) and 100 cc. ether. Shake thoroughly and permit the two layers to separate; draw off the lower aqueous layer into a second 1000-cc. separatory funnel; retain the upper ether layer. Extract the aqueous layer a second time with 100 cc. of ether; the aqueous layer should now be colorless. Combine the two ether layers, dry with 25 Gm. of anhydrous sodium sulfate and filter; wash out any oil adhering to the sodium sulfate with a 25-cc. portion of ether and add this washing to the dried ether solution. Evaporate the ether in a Pyrex evaporating dish which has a top diameter of 90 mm. and a height of 50 mm. The evaporating dish should be heated on a steam bath in such a manner that only the flattened bottom is in contact with the steam; a few small clay chips should be added to prevent bumping and to hasten evaporation. Add initially about 75 cc. of the ethereal solution to the dish, and when this volume is reduced to about 50 cc., add a further 25-cc. portion of the ethereal solution. After all of the solution has been added in this manner, continue the evaporation until the thin small stream of ether bubbles rising from the clay chips just ceases. Continue the heating on the steam bath for an additional thirty minutes; remove the evaporating dish and pour the contents into a test tube. Determine the behavior of the separated phenols at various fixed temperatures, using the technique described under the paragraph "Procedure" in Part A.

The phenols separated from the eleven samples behaved as indicated in Table IV.

It should be noted that the phenolic mixtures recovered from the reconstituted thyme oils (Table IV) behave somewhat differently from the original mixtures (Table II). This variation in behavior is due to many contributing factors, some of which are discussed below.

1. The complete removal of the ether from the phenols is essential as evidenced by the following experiment. About 5 Gm. of thymol (congealing point = +49.4°, when supercooled to +40° and seeded) was dissolved in 100 cc. of ether, and the ether removed by the technique described in the procedure for the separation and recovery of the phenols from thyme oil. When the stream of ether bubbles just ceased, the evaporating dish was removed from the steam bath and permitted to cool; no odor of ether was observed. When cooled to 40° and seeded, no crystallization occurred; when cooled to +30° and seeded, crystallization occurred with a rise of temperature to only 35.5°. The evaporating dish was then permitted to stand open overnight to permit the residual ether to evaporate; when seeded at a temperature of +35°, a congealing point of 41.8° was obtained. Additional heating for 10 minutes raised the congealing point to +48.0° when seeded at +40°. The experiment was repeated, but the evaporating dish was heated for a period of thirty minutes after the stream of ether bubbles just ceased; a congealing point of +49.2° was obtained when seeded at +40°. Hence this thirty-minute heating period was incorporated in the procedure.

2. The heating period of thirty minutes alters the composition of the phenolic mixture because of a difference in the vapor pressure of thymol and carvacrol. This factor affects somewhat the accuracy of the experimental results.

About 10 Gm. of a mixture of 70% thymol–30% carvacrol was heated in a glass evaporating dish as described in the procedure. The loss of weight and the congealing point were determined after thirty minutes and after sixty minutes. The following data were obtained.

	Original	After 30 Min.	After 60 Min.
Total loss	....	3.3%	6.4%
Congealing point, supercooled to +30° and seeded	+33.1°	+32.3°	+31.3°

3. The alkaline solution used for the separation of the phenols has a definite adverse effect upon thymol.

[1] The solution is not washed with ether at this point; this is to prevent loss of the phenols, since both carvacrol and thymol can readily be washed out of an alkaline solution of their salts with ether. This fact has been reported in the literature (11) and was confirmed by the author.

Upon dissolving 12.5 Gm. of thymol in 250 cc. of potassium hydroxide T.S. a clear colorless solution resulted. Upon standing overnight, this solution developed a yellow color but remained clear. Upon acidification and subsequent recovery of the thymol, 12.2 Gm. of a deep yellow colored material was obtained. The behavior of the recovered thymol, compared with the behavior of the original material, is shown below:

	Congealing Point	
	Original	Recovered
Seeded, after being supercooled to:		
30°	49.0°	43.8°
35°	49.2°	44.7°
40°	49.4°	45.6°

4. Any acids that are present in a thyme oil might interfere with the determination of thymol unless they are removed by a preliminary treatment. The free acids of two normal Spanish oils were separated; the amount present is too small to offer any appreciable interference.

TABLE III.—RECONSTITUTED THYME OILS

No.	Composition, % Thymol	Carvacrol	Nonphenols	$d_{25°}^{25°}$	$\alpha_D^{25°}$	$n_D^{20°}$	Sol. 25°	Phenol Content (Vol., %)	Relative Viscosity at 22°[a]
A0	0	50	50	0.928	−1°26′	1.5020	Clearly soluble in 2–2.5 vols. 70% alcohol and more	48.4	1.6
A1	5	45	50	0.928	−1°26′	1.5018		48.0	...
A2	10	40	50	0.927	−1°26′	1.5016		48.0	...
A3	15	35	50	0.927	−1°26′	1.5013		48.0	...
A4	20	30	50	0.927	−1°26′	1.5013		48.0	...
A5	25	25	50	0.927	−1°26′	1.5012		48.0	1.6
A6	30	20	50	0.927	−1°26′	1.5012		48.0	...
A7	35	15	50	0.926	−1°26′	1.5012		48.0	...
A8	40	10	50	0.926	−1°26′	1.5010		48.0	...
A9	45	5	50	0.926	−1°26′	1.5009		48.0	...
A10	50	0	50	0.926	−1°26′	1.5008		47.8	1.6

[a] Determined by means of a pipette; viscosity of distilled water = 1.00.

TABLE IV.—BEHAVIOR OF RECOVERED PHENOLS

No.	Original Composition, % Thymol	Carvacrol	Mixture Cooled to 0°	5°	10°	15°	20°	25°	30°	35°	40°	45°	50°
A0	0	100	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
A1	10	90	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
A2	20	80	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
A3	30	70	C.S. Note 4	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
A4	40	60	C.S. Note 4	C.S. Note 4	C.S. Notes 1 & 3	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
A5	50	50				C.S. Note 4	C.S. Note 1	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
A6	60	40					C.I. C.P. = 23.2°	C.I. Note 1	N.C.	N.C.	N.C.	N.C.	N.C.
A7	70	30					C.I. C.P. = 26.7°	C.I. C.P. = 29.8°	C.S. Note 1	N.C.	N.C.	N.C.	N.C.
A8	80	20						C.I. C.P. = 33.0°	C.I. C.P. = 35.3°	C.I. C.P. = 36.4°	N.C.	N.C.	N.C.
A9	90	10							C.I. C.P. = 39.5°	C.I. C.P. = 40.3°	C.I. C.P. = 41.5°	N.C.	N.C.
A10	100	0							C.I. C.P. = 43.8°	C.I. C.P. = 44.7°	C.I. C.P. = 45.4°	C.S. Note 1	N.C.

Key: N.C. = no crystallization observed; C.I. = crystallizes immediately; C.S. = crystallizes slowly; C.P. = congealing point.

NOTE 1—No appreciable temperature rise observed.
NOTE 2—Mixture becomes turbid owing to the presence of thymol crystals.
NOTE 3—Mixture becomes hazy owing to the presence of thymol crystals.
NOTE 4—Temperature rise is slow; congealing point is not sharp and is not easily reproducible.

Approximately 100 cc. of thyme oil was shaken thoroughly with 25 cc. of sodium carbonate T.S.: no liberation of carbon dioxide was observed. After twenty-four hours, the aqueous layer was separated and washed with three 25-cc. portions of ether to remove any dissolved or suspended oil. After acidification with 25 cc. of dilute sulfuric acid (1:3), the liberated acids were extracted with 50 cc. ether. Upon evaporation of the dried ether layer, a small amount of a dark oily liquid with the

characteristic odor of the lower fatty acids was obtained. The amount recovered weighed 0.012 Gm. and 0.016 Gm. for the two thyme oils used in this experiment.

DISCUSSION

A study of Table II shows that a mixture of the two phenols containing 20% thymol will show no crystal formation within five minutes when cooled to 0° and seeded. The 30% thymol sample, however, crystallizes slowly at 0° as well as at 5°; no appreciable temperature rise is observed, however. The 50% thymol sample is the first to show a decided rise in temperature during the crystallization. In this case the congealing point is obviously dependent upon the degree of supercooling; when supercooled to 5°, the congealing point was 15.5°; when supercooled to 15°, the congealing point was 20.5°. As the percentage of thymol is increased from 50% to 100%, the congealing points become sharper and more easily reproducible. However, even the 100% thymol sample showed some variation in congealing point depending upon the degree of supercooling.

Sage and Dalton (7) reported values in disagreement with these data. Unfortunately, these authors did not give constants for the carvacrol used in their experiments; possibly the carvacrol contained thymol, since it is frequently obtained in commerce as a by-product in the manufacture of thymol. It is interesting to note tnat these investigators reported no difficulty was encountered in determining congealing points, even with mixtures containing but 10% thymol. Furthermore, no depression of congealing point was reported for pure carvacrol when admixed with small percentages of thymol.

In Table IV it will be seen that a convenient change in behavior occurs between 40% and 50% thymol; i.e., separated phenols consisting of 50% thymol crystallize at 20° (normally considered room temperature), but a temperature below 15° is required for crystallization of the separated phenols consisting of 40% thymol. Furthermore, this same relationship exists for mixtures of the pure phenols (see Table II).

The congealing points reported by Sage and Dalton (7) for recovered phenols are in disagreement with the data given in Table IV; again the possibility that their carvacrol contained thymol cannot be overlooked.

The greatest difference in behavior of the mixtures of pure phenols and the phenols recovered from the reconstituted thyme oils occurs at high thymol percentages. This is to be expected, since the melting point of a pure substance (thymol) is lowered considerably more by a small amount of impurity than is the melting point of a mixture (50% thymol–50% carvacrol). However, if some arbitrary value is selected as a criterion for a thymol type of oil, this factor will be minimized, provided that the arbitrary value is fixed at a low figure; for example, 50% thymol.

The procedure described requires the use of 25 cc. of oil. This amount was chosen so that approximately 10 Gm. of recovered phenol would be available for investigation. However, if such a quantity of oil is not available the aqueous alkaline solution from the official determination of total phenols may be used. The quantities of solutions and reagents employed should be reduced proportionately. The recovered phenols in such cases amount to approximately 4 to 6 Gm., depending on the total phenol content of the oil. The behavior on seeding at various fixed temperatures is in close agreement with the values reported for the 10-Gm. samples.

SUMMARY

A procedure has been described for the separation of phenols from thyme oils; the behavior of known mixtures of such separated phenols at fixed temperatures when seeded with a crystal of thymol has been described.

If the phenols separated from Spanish thyme oil crystallize at room temperature (20°) after seeding, the phenols of the original oil consist of more than 40% thymol; these oils cannot be of the carvacrol type. Such behavior is indicative that the oil is primarily of the thymol type, official in the National Formulary, Eighth Edition.

Furthermore, if the separated phenols crystallize within five minutes when cooled to 0° and seeded, the phenols of the original oil contain more than 20% thymol.

The experimental part of this paper follows the general outline of the work of Sage and Dalton (7); however, the results are at variance to the data presented in this earlier work. The discrepancies may well be due to the fact that the carvacrol employed by Sage and Dalton may have contained thymol as an impurity.

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Improved Methods of Tablet Coating*

By JOSEPH A. KOREN and BYRL E. BENTON

This paper describes an attempt to develop a standardized method of tablet coating; i.e., the application of well-defined procedures of precise quantities of liquids and solids to a definite weight or volume of tablets. Experiments presented show that the standardization of tablet coating is possible. The disintegration tests show that the tablets coated by the outlined processes disintegrate rapidly.

COATING of solid medicinals has been practiced for a long time and the operation has been regarded as one of the mystic arts (1). Stokes (2) and Chilson (3) point out that the processes and techniques employed in the coating of tablets have been surrounded with more or less secrecy. Tablet coating, according to Chilson (4), is one of the most variable processes in the pharmaceutical industry; it is wholly governed by rule-of-thumb methods and has in consequence led to a variety of formulas and methods. According to the information found in the literature, the general opinion seems to be that tablet coating is an "art" which can be acquired by practice and experience rather than by the use of accurate weights and measures and standard procedures.

The need for further study on tablet coating has been shown by the numerous and various methods now employed and by the lack of adequate information reported in the literature regarding a standard procedure. This paper describes an attempt to develop a standardized method of tablet coating; i.e., the application by well-defined procedures of precise quantities of liquids and solids to a definite weight and volume of tablets. It is especially important that the liquids and solids be combined in a definite ratio, thereby eliminating the guesswork in the most variable operation of tablet coating. The conventional methods were studied and improved methods were established; these methods were then applied and the results recorded.

EXPERIMENTAL

The equipment used in these investigations consisted of a 20-inch galvanized iron coating pan and a 20-inch canvas-lined galvanized iron polishing pan. These two pans were interchangeable on the same motor-driven bench stand. The motor was geared down so that the pan on the shaft made 36 r. p. m.

The hot air blast was supplied by the exhaust and the vacuum by the suction of a commercial vacuum cleaner. The hot air blast was used simultaneously with the vacuum at all times during the coating process. After the vacuum cleaner had been running for approximately twenty minutes the temperature of the hot air blast reached 55°. The hot air exhaust was directed at the upper end of the pan, while the suction end was placed slightly above the tablets. The position of the two ends created a constant air current, heated the pan, and removed free dust or powder that was given off by the tablets during the drying process.

A hot plate and a water bath supplied the heat necessary for heating the coating liquids during the coating operation.

A Waring Blendor with blades revolving at a speed of 10,000 r. p. m. was used for mixing all the suspensions before they were applied to the tablets. The glass container of the Waring Blendor was graduated so that the suspensions could be accurately measured.

The Hardness Tester for Tablets furnished by the Monsanto Chemical Company of St. Louis, Mo., was used to determine the degree of hardness of the tablets.

The disintegration of the tablets (uncoated and coated) was determined by an instrument employed by Granberg (5). A constant water bath with a temperature of 37° was used to maintain the acid-pepsin solution (6) at body temperature during the disintegration tests.

Three thousand nine hundred grams of four-grain placebo tablets (approximately 15,000 tablets) were employed in all the coating operations. The placebo tablets contained 10 parts of lactose and 1 part of starch which was mixed with $1^1/_2$ parts of starch paste (10% *w/v*) and granulated through a No. 12 sieve. After the granules were dry, 4% starch, 2% talc, and 0.5% magnesium stearate were added to facilitate the compression of the tablets. The tablets were compressed on a Stokes model "E" single-punch machine using deep concave $^{11}/_{32}$-inch punches and die. The pressure was adjusted so that the tablet hardness was between 3 and 4 Kg.

The following formulas were employed in these investigations (Table I). All of the ingredients are of U. S. P. or N. F. quality unless otherwise indicated.

The shellac solution used was the full strength Nu-Pros Glaze manufactured by James B. Day Company of Chicago, Ill. The shellac solution contains 4 lb. of arsenic-free shellac in sufficient alcohol to make one gallon.

The classification of the operations in the process of coating tablets varies among the different men

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with experience in coating. The coating employed in these experiments has been divided into the undercoat, the subcoat, the syrup coat, the finishing coat, and the polishing coat.

During the undercoating process, it was found that 150 cc. of the shellac solution was sufficient to give satisfactory results.

In the first experiment of the subcoating process the same amount of the subcoating solution (Formula 1) was added for each application (Table II). Sufficient powder (Formula 2) was then applied to the tablets to prevent excessive sticking. The powder was in a tared vessel which was weighed before and after each dusting. It was noticed that if a particular application required a great quantity of powder the next application would require less powder and vice versa. An analysis of the quantities of dusting powder used with a constant amount of subcoating solution indicated that there was a relationship between the amount of subcoating solution and the amount of dusting powder.

In the next two experiments, the quantity of the subcoating solution was reduced after the first application and then kept constant in subsequent applications. It was decided to decrease gradually the amount of the dusting powder to avoid the accumulation of an excessive amount of free powder.

During the first three experiments it was noticed that the amount of dusting powder required to prevent the tablets from becoming excessively sticky also depended upon when the powder was added to the tablets.

To eliminate the guesswork as to when to add the powder and how much powder to add for a smoother subcoat and to reduce the time required for tablet coating, the dusting powder was incorporated in the subcoating solution in the fourth experiment. Various quantities of this subcoating suspension (Formula 3) were applied.

The results obtained in the fourth experiment indicate that a combination of dusting powder and subcoating solution applied as a suspension gives satisfactory results.

TABLE I.—PERCENTAGE COMPOSITION OF THE FORMULAS EMPLOYED IN TABLET COATING

Ingredient	1	2	3	4	5	6	7	8	9	10	11	12	13
Gelatin	3.5	..	..	10	5	..	3						
Acacia	3	4	..	10	5	..	3						
Sugar	55	72	..	50	50	85							
Talc	..	4	..	10	25	..	..	5	10	5			
Starch	..	20	..	10	5	..	..	15	30	15			
Formula 1	..	..	50										
Formula 2	..	..	50										
Formula 6	..	..	..	..	..	..	94		60	80	50		
Formula 7	..	..	..	..	..	..	..	80					
White wax	..	..	..	..	..	..	..	..	..	..	..	67	
Carnauba wax	..	..	..	..	..	..	..	..	..	..	..	33	
Formula 12	..	..	..	..	..	..	..	..	..	..	..	..	5
Naphtha (commercial grade)	..	..	..	..	..	..	..	..	..	..	..	..	95
Dist. water	38.5	..	..	10	10	15	..	..	..	..	50	..	
Total	100	100	100	100	100	100	100	100	100	100	100	100	100

Formulas 1, 3, 4, and 5 were applied at a temperature of 70°. Formula 2 was sifted through a No. 80 screen. Formulas 6, 7, 9, 10, and 11 were applied at a temperature of 20°. Formulas 8 and 13 were applied at a temperature of 50°. Formula 12 was melted, mixed well, and allowed to solidify.

TABLE II.—QUANTITIES APPLIED DURING THE SUBCOATING PROCESS

Experiment	Formula Used	Application* 1	2	3	4	5	6	7	8	9	10	11	12
1	1	75	75	75	75	75	75	75					
	2	30	15	60	20	40	35	40					
2	1	75	50	50	50	50	50	50	50				
	2	60	30	30	15	15	15	15	30[b]				
3	1	60	50	50	50	50	50	50	50	50	50		
	2	40	35	30	25	25	25	20	20	15	0		
4	3	60	120	100	75	75	75	120	75	75	75		
5	4	100	60	25	40	15	20						
	5	..	..	..	..	..	..	40	60	65	60	60	80
6	8	60	50	50	60	75	100	125	150	200			
7	8	50	65	80	125	150							
8	8	60	100	150	175	150	150	150					
9	10	60											
	8	..	50	75	100	125	150	150	175	175	175	200	
10	9	60											
	8	..	75	75	100	125	150	150	150	175	175	200	175

[a] Solids weighed in grams. Liquids measured in cubic centimeters.
[b] The amount of the dusting powder was increased because the tablets were not filling out.

In an attempt to obtain a more favorable ratio between the powder and the liquid, the subcoating mixture No. 1 (Formula 4) and the subcoating mixture No. 2 (Formula 5) were employed in the fifth experiment.

The results obtained from the applications of the subcoating mixture No. 1 indicated that too much adhesive substances and not enough insoluble powders were present; therefore, subcoating mixture No. 2 was formulated.

Since the subcoating mixtures Nos. 1 and 2 employed in the fifth experiment were unsatisfactory a new compound subcoating mixture (Formula 8) was employed in the sixth experiment. With the exception of the ninth application, gradual increase of the amount of the mixture gave satisfactory results. Too much of the mixture was added in the ninth application and the tablets became excessively sticky. The compound subcoating mixture gave satisfactory results until an excess amount was added. It was therefore decided to employ it in the seventh experiment.

As in the previous experiment, the first application of the compound subcoating mixture (Formula 8) in the seventh experiment (even though the quantity was reduced) caused the tablets to stick to the side of the pan. However, when the second application was added, although the amount was increased, the tablets did not become excessively sticky. In fact, all of the subsequent applications gave satisfactory results. It may be possible that the excessive sticking after the first application may be due to the shellac undercoat plus the moisture content of the subcoating liquid. To test this hypothesis the undercoat was omitted in the eighth experiment and the compound subcoating mixture was applied directly to the tablets. The tablets did not stick excessively; however, they did become tacky and had pinholes in them where they had pulled apart from each other. The amount of the compound subcoating mixture was increased in the next two applications without any excessive sticking, but the amount of the mixture in the fourth application caused the tablets to stick excessively. The sticking after the fourth application was due to the addition of an excessive amount of the mixture. The amount of the mixture was reduced in the subsequent applications and satisfactory results were obtained.

The undercoat was applied as usual in the ninth experiment because it was found to give a better foundation for subsequent coats. To prevent excessive sticking when the first application of the compound subcoating mixture was applied, the syrup mixture No. 2 (Formula 10) was employed after the undercoat. The syrup mixture No. 2 made the tablets very tacky; however, the tablets did not stick to the side of the pan. The first application of the compound subcoating mixture did not cause the tablets to stick excessively; on the contrary, the tablets became only slightly tacky. The amount of the compound subcoating mixture was gradually increased in the subsequent applications and in each case the action of the tablets was satisfactory. These results indicate that the application of a syrup mixture before subcoating a tablet may be desirable.

Although the tablets did not stick to the side of the pan when the syrup mixture No. 2 was applied in the ninth experiment, they did become very tacky. Therefore, a priming mixture (Formula 9) was formulated and employed in the tenth experiment. This mixture was applied after the undercoat. There was no excessive sticking either with the priming mixture or with the first application of the compound subcoating mixture. The quantity of the compound subcoating mixture was gradually increased in the subsequent applications until the eleventh application. The tablets became excessively sticky after the eleventh application; therefore, the amount was decreased in the last application.

Various amounts of syrup solution (Formula 6) and syrup mixtures (Formula 10) (Table II) were applied in each experiment in an attempt to obtain the proper quantity to be used for a definite weight of tablets. It was found that 60 cc. of syrup mixture No. 2 and 40 cc. of syrup solution gave satisfactory results.

During the finishing process it was found that 15 or 20 cc. of the finishing solution (Formula 11) could be applied to the tablets without causing them to stick excessively.

During the polishing process it was found that one application of 60 cc. of the wax solution (Formula 13) was sufficient to impart a satisfactory luster to the tablets.

Several *in vitro* methods have been suggested for determining the relative rate of disintegration of the tablets. The procedure employed by Granberg (5) was selected for this study. Ten uncoated and ten coated tablets from each experiment were tested to give an average disintegrating time (Table III).

TABLE III.—AVERAGE DISINTEGRATION TIME IN MINUTES AND SECONDS

Experiment	Uncoated Tablet	Coated Tablet
1	13 sec.	9 min., 35 sec.
2	14 sec.	8 min., 34 sec.
3	12 sec.	2 min., 50 sec.
4	10 sec.	3 min., 41 sec.
5	10 sec.	2 min., 37 sec.
6	12 sec.	2 min., 35 sec.
7	12 sec.	1 min., 30 sec.
8	12 sec.	2 min., 18 sec.
9	12 sec.	2 min., 32 sec.
10	29 sec.	4 min., 10 sec.

SUMMARY AND CONCLUSION

1. An undercoat of shellac is desirable as a foundation for subsequent coatings to produce smooth tablets.

2. A priming coat consisting of insoluble powders suspended in syrup solution aids in producing a smooth tablet.

3. The incorporation of insoluble powders in the subcoating solution facilitates their application to the tablet and at the same time produces a smoother subcoat.

4. The amounts of syrup mixture, syrup solution, and finishing solution have been standardized for a given weight of tablets.

5. Tests show that the tablets coated by the outlined processes disintegrate rapidly.

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A Contribution to the Pharmacology of 5-Nitroguaiacol*,†

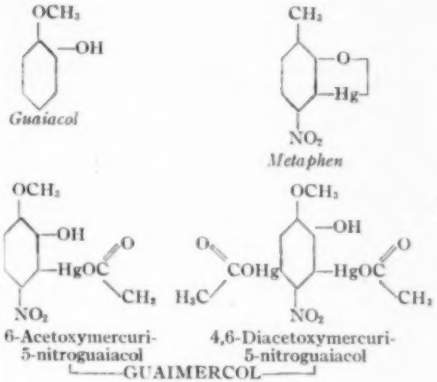
By NORMAN W. PINSCHMIDT‡ and JOHN C. KRANTZ, JR.

***In vitro* tests on mercurated 5-nitroguaiacol indicate that it may be an effective antiseptic, both in its solid form and in solution as the sodium salt. The results of toxicity studies are reported.**

THE ORGANIC mercurial antiseptics are, for the most part, monomercury substitution products of benzene and its derivatives. In a study concerned with the mercuration of the benzene nucleus certain derivatives of guaiacol were made available. Of the many mercury compounds of guaiacol and its derivatives which were synthesized in this study, the compound obtained by direct mercuration of 5-nitroguaiacol appeared to be the most promising for pharmacologic study. Owing to the former widespread empirical use of guaiacol in pulmonary infections, we decided to investigate this compound.

In a study of the structure of these mercury derivatives of 5-nitroguaiacol, Drake, Harris, and Jaeger (1) have shown that direct mercuration of 5-nitroguaiacol with mercuric acetate results in a product having a mercury content of 49 to 54 per cent (depending upon the exact mercuration conditions) and that it consists of, principally, 6-acetoxymercuri-5-nitroguaiacol and 4,6-diacetoxymercuri-5-nitroguaiacol. The product is very insoluble in water and organic solvents, with the exception of acetic acid, and does not have a clearly defined melting point. It has resisted every effort to separate it into pure components. It is available under the name of "Guaimercol."

The acetoxymercuric derivative of 5-nitroguaiacol used in this study is a yellow, finely divided powder fluorescing as brick-red under ultraviolet light. It has a mercury content of 53.8 per cent. Its relationship to guaiacol and "Metaphen" is apparent from the following formulas.



Strong bases form the corresponding soluble phenolates of the compound. Each 100 mg. of the powder is therefore treated with 2 cc. of 1 *N* sodium hydroxide before dilution with water. The 1:1000 solution, thus prepared, although strongly alkaline (*p*H 10.5 to 11) is suitable for intravenous injection if administered slowly. Irrespective of treatment by various buffers and protective colloids, solutions of Guaimercol are unstable at a *p*H below 10.5. The alkaline solution is unstable in air but if preserved under nitrogen in sealed glass ampules, it will resist extreme conditions of light, temperature, and agitation.

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TOXICITY STUDIES

In establishing the values given in Table I, the method of Behrens (2) for deduced mortalities was used, the LD_{50} being obtained by graphing.

In the case of the intraperitoneal injection in rats a comparison has been made between young and older animals. The factors of weight and age seem to have little effect in the toxicity of this compound.

Representative animals of the higher dose ranges used in establishing the LD_{50}'s were autopsied and microscopic studies were made on their tissues. The typical lesions of mercurial poisoning were found in the kidneys and colon. In addition, some subcapsular necrosis was found in the liver. All other organs appeared normal.

In order to establish the chronic or cumulative toxicity, the compound was administered in one-half the minimum lethal dose (0.5 mg./Kg.) to a series of five rabbits. The drug was given intravenously twice weekly for a period of six weeks. At the end of this period the animals were sacrificed and liver, kidney, and colon sections were examined microscopically. In no instance was there any symptom of mercurial poisoning other than a slight cloudy swelling of the proximal tubules of the kidney. Two of the animals developed late in the study a severe diarrhea and loss of weight and finally expired. Their pathology, however, did not in any way resemble that of mercurial poisoning, one apparently dying of an infection of the large bile ducts and the other of unknown causes. The other animals remained healthy and normal throughout the experiment and showed a normal weight increase.

A further study of the cumulative toxicity was made on a series of 12 dogs to which was administered an intravenous dose of 0.5 mg./Kg. on five successive days. The animals were biopsied in from three to five days following the last injection and histological examinations made of their livers and kidneys. With the exception of a mild swelling of the tubules with little or no debris in the lumina, the kidneys were normal. The livers showed in several cases a cloudy swelling of a diffused nature and in one case some slight degeneration of the liver cells. As those changes are reversible and of such a mild nature, they are not considered significant of chronic irreparable damage.

Bromsulfonphthalein Test for Liver Function.—Six dogs were tested for normal liver function according to the standard values used in this laboratory, i.e., that less than 3% of the dye remains in the blood stream thirty minutes after injection. The animals were then injected intravenously on each of five successive days with 0.5 mg./Kg. Guaimercol. After two days the bromsulfonphthalein test was repeated for three successive days on each animal. No deviation from the normal was observed.

Phenolsulfonphthalein Test for Kidney Function.—Six dogs were injected for five days with an intravenous dose of 0.5 mg./Kg. of Guaimercol.

After two days rest the dye was administered by intramuscular injection and overnight (eighteen hours) urine samples collected. These samples showed an elimination of the dye in amounts comparable to values on normal dogs.

TABLE I.—COMPARATIVE TOXICITY OF GUAIMERCOL WITH VARIOUS MODES OF ADMINISTRATION: COMPARATIVE TOXICITY WITH METAPHEN

Compound	Animal	No. Animals	Wt., Gm.	Mode of Administration	LD_{50} (Mg./Kg.)
Guaimercol	Albino rats	42	140–160	Intraperitoneally	6.0
	Albino rats	36	180–210	Intraperitoneally	6.9
	Albino rats	54	180–250	Orally	125.0
	Rabbits	42	1500–2400	Intravenously	2.6
Metaphen	Albino rats	36	140–160	Intraperitoneally	3.0

In order to confirm these findings, the dogs were further tested at the time of the biopsy for the collection of kidney and liver specimens (above). After the removal of one kidney under pentobarbital anesthesia, the ureter of the remaining kidney was cannulated and the urine flow adjusted, by means of intravenous injection of 50% glucose, to from 7 to 10 drops per minute. Urine was collected over a period of two and one-half hours and made up to 500 cc. for comparisons with U. S. P. standards. The values obtained (45 to 55% dose) compared favorably with those obtained on normal dogs by the same method.

Reaction with Normal Body Constituents.—Tests were run with Guaimercol against a representative sulfur-bearing compound, cysteine hydrochloride. Solutions of Guaimercol 1:1000 were apparently unaffected even upon long standing (seventy-two hours) by an equal volume of cysteine hydrochloride solution 1:1000.

Rabbit, horse, and human serum in concentrations ranging from 10 to 50% were mixed with Guaimercol 1:1000, Mercurochrome 1:50, Metaphen 1:1000, and mercuric chloride 1:1000 and allowed to stand at 37° for twenty-four hours. Only in the case of mercuric chloride was there any great degree of precipitation. The three organic mercurials showed no greater precipitation of serum than was caused by an equal volume of distilled water.

Guaimercol and mercurochrome were found to be soluble in normal horse serum.

Respiration Studies.—Studies on Guaimercol were conducted by Dr. Wilson C. Grant of this laboratory using the Warburg technique for the study of the respiration of isolated tissue sections.

The mercurated 5-nitroguaiacol compound in the concentration employed caused a depression in the respiratory quotient of kidney and tumor tissue only.

It may be calculated that the concentration of mercurated 5-nitroguaiacol used in this test is ap-

proximately that which would appear in the blood of an animal receiving a dose of 6.6 mg./Kg. of the drug. Reference to the toxicity table previously given shows this to be a fatal dose (corresponding with the LD_{50} in rats).

PHARMACODYNAMIC STUDIES

Blood Pressure.—Stefano and Querico (3) have shown that guaiacol injected intravenously into higher animals is a blood pressure reductant. Guaimercol, however, did not alter the blood pressure of dogs under ether anesthesia at any of a wide range of doses. As much as 30 cc. of a 1:500 alkaline solution failed to produce any change in 5 medium-sized dogs injected.

Cardiac Toxicity.—It has also been shown by Stefano and Querico that guaiacol causes a depression of the isolated frog's heart that is sometimes irreversible. In the frog heart perfusion (thirteen minutes) with a 1:100,000 solution of mercurated 5-nitroguaiacol in Howell-Ringer's solution, there was no change in amplitude, rhythm, or time of the heart beat.

Diuresis.—A series of six dogs was studied in which the ureters were cannulated and the urine flow measured before and after administration of large doses (10 cc. of 1:1000 alkaline solution to 8 to 10 Kg. dogs). In no case was there any significant increase in the flow of urine.

Bronchiolar Action.—The compound was perfused through the freshly isolated lungs of the guinea pig. The degree of bronchiolar dilatation or constriction was measured by counting the number of drops of saline and drug as they perfused through the organ. No significant change was observed in any of the preparations after addition of Guaimercol 1:1000 and 1:10,000 to the perfusion solution.

BACTERIOLOGICAL CONSIDERATIONS

The antiseptic potency of Guaimercol was established using the simplified Kolmer technique (4).

A $HgCl_2$ coefficient was established by dividing the denominator of the highest dilution of Guaimercol producing inhibition of each organism with the corresponding value for mercuric chloride. The results are shown in Table II.

TABLE II.—MERCURATED 5-NITROGUAIACOL VS. PATHOGENIC ORGANISMS (RESULTS AFTER FIVE DAYS OF INCUBATION)

Organism	Protection in Dilutions	$HgCl_2$ Coeff.
Staphylococcus aureus	Up to 1:960,000	27.3
Eberthella typhi	Up to 1:160,000	2.0
Flexnor bacillus	Up to 1:320,000	1.0
B. coli	Up to 240,000	0.69

Reaction with Body Fluids.—In order to approximate the action of the compound under conditions similar to those in living tissues, the antiseptic was similarly tested in varying dilutions with solutions containing 10% normal serum, 10% ascitic fluid, and 10% whole blood. The test organism was the standard *E. typhi* strain. With Guaimercol and three other organic mercurials studied, the range of protection was reduced to half by the presence of serum and ascitic fluid. The mercuric chloride was almost completely inactivated by the presence of these fluids.

With 10% serum we observed a very slight growth even in the highest concentrations attainable of all the mercurials. As this growth did not increase on standing and as 3 drops of solution plated on agar showed, in every case, less than 50 colonies, it was interpreted that there was a partial killing and bacteriostasis of the organism in the presence of serum.

In the presence of a 10% defibrinated human blood the mercurated 5-nitroguaiacol showed no bacteriostasis in concentrations up to 1:10,000 (a relatively high concentration in terms of the normal potency of the compound).

Cup and Pour Plate Tests.—Using the standard cup and pour plate methods (with *E. Typhosa* and *S. Aureus*), there was observed a large area of clearing with the mercurated 5-nitroguaiacol. Of particular interest is the fact that the compound in its solid form, notwithstanding the fact that it is so insoluble in water, was toxic to organisms within a radius of 2 to 5 cm.

CONCLUSIONS

1. Some LD_{50}'s have been established for mercurated 5-nitroguaiacol which consists of, principally, 6-acetoxymercuri-5-nitroguaiacol and 4,6-diacetoxymercuri-5-nitroguaiacol. The compound is least toxic when administered by mouth.

2. Mercurated 5-nitroguaiacol has been shown to be administrable by vein in repeated sublethal doses without significant tissue damage.

3. When administered in lethal doses, it has been shown to produce damage primarily in the kidneys, colon, and liver, death apparently ensuing as a result of typical mercurial nephrosis. Lethal doses also produce an inhibition to the respiration of kidney and tumor.

4. *In vitro* tests show it to be an effective antiseptic of the order of 4-nitro-5-hydroxymercurithocresol (Metaphen-Abbott). It exhibits antiseptic properties both in its solid form and as the soluble sodium salt.

5. It has been shown to be without effect on blood pressure, heart action, urine output, or bronchiolar tone of animals.

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Preliminary Report On Microamorphous Silica as a Suspending Agent in Calamine Lotion*

By C. LEE HUYCK†

Two and four per cent aqueous suspensions of microcrystalline silica are described. These suspensions were substituted for 5 per cent aqueous bentonite suspension in Calamine Lotion and Chalk Mixture. Four per cent silica suspension appears to be superior to 5 per cent bentonite suspension in Calamine Lotion. Substitution of 5 per cent bentonite by 4 per cent silica suspension in Chalk Mixture proved to be unsatisfactory.

SPECIAL SOAPS containing silica (1) are made by treating a soap containing sodium silicate with a solution of phosphoric acid.

Silikat-Hautsalbe (2) is an ointment of lanolin and silicic acid used for frostbite, skin eruptions, pimples, red nose and face. Silikat-Milchpuder is a dusting powder containing silicic acid in an easily absorbed form indicated for wound, burn, skin itch, wet eczema, and inflammation.

Brock (3), *et al.*, prepared a silica gel suitable for cosmetic preparation by introducing silicon tetrafluoride into an aqueous solution of fluosilicic acid. Heuser used this form of silica gel as a dentifrice.

Leone and Sante (4) reported on the toxicity of pure, stable 0.5 per cent colloidal silica. When administered parenterally, colloidal silica exerts a depressing action in small doses and a paralyzing action at high doses.

Silantox (5) is a colloidal silica used internally as an intestinal absorbent and externally as a dusting powder.

Peronnet and Genet (6) prepared silica gel from sodium silicate and hydrochloric acid. It was filtered on a Buchner filter, washed and mixed with glycerin to give a gel having a consistency of petrolatum. Formulas are given for utilizing this gel as an ointment base. The ointment may be washed from the skin with water. No report is made on its therapeutic efficacy.

Belcot (7) published formulas of silica gel containing cosmetics and medicinal preparations.

Prout, Eddleman and Harris (8) investigated the value of such a base as a carrier for germicidal substances. They found that zinc oxide, boric acid, phenol and ammoniated mercury in a silica gel-glycerin base had greater zones of inhibition than the ointments of germicidal substances official in the United States Pharmacopoeia. The true value of this base, however, must await clinical evaluation.

Prout and Harris (9) reported on the emulsifying properties of silica gel and formulated a base consisting of 45 per cent silica gel, 5 per cent hydrous wool fat, 15 per cent liquid petroleum, and 35 per cent petrolatum.

Herfurth and Zellwalle (10) found that an emulsion of viscous petroleum oil and silica gel is suitable for use in ointments, creams, emollients, and soap mixtures.

Redgrove (11) referred to a patent by Heuser in which light amorphous silica is used to make excellent face powder bases. These contain about 20 per cent silica. In addition to being light, and fluffy, the silica possesses excellent covering power and a remarkable capacity for absorbing moisture. In the two latter respects it is definitely superior to kaolin and precipitated chalk. While some felt that the silica might irritate the skin, the author felt that the use of colloidal or amorphous silica is without danger. In powder creams, amorphous silica gave exceptionally good results but was unsuitable in liquid powders because of its tendency to form hard gels.

EXPERIMENTAL

Microamorphous silica,[1] of a maximum particle size of 50 mμ was used. It has a linseed oil absorption value of 400, density of 2.2, index of refraction 1.46, purity of 99.9% and 8% absorbed water. Two and 4% aqueous suspensions were prepared by heating distilled water to boiling and adding the silica gradually with constant stirring. These suspensions were allowed to stand for twenty-four hours and then stirred vigorously again. The suspensions were substituted for 5% aqueous bentonite suspension in two pharmaceutical preparations requiring suspending agents, namely Calamine Lotion, and Chalk Mixture of the U. S. P. When 5% bentonite suspension was used in Calamine Lotion the average amount of water which separated on the surface of duplicate samples after forty-eight hours was 15 cc. per 85 cc. of finished product.

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[1] Silica was furnished through the courtesy of the Linde Air Products Co., 30 E. 42nd St., New York 17, N. Y.

When 2% silica suspension was used as a suspending agent the average amount of water which separated from duplicate samples was 20 cc. per 90 cc. of finished product. When 4% silica suspension was substituted for the bentonite suspension the average amount of water which separated on the surface of duplicate samples was 7 cc. per 92 cc. of finished product. In Calamine Lotion, 4% silica suspension appeared to be superior to 5% bentonite suspension. When 4% silica suspension was substituted for 5% bentonite suspension in Chalk Mixture of the U. S. P., the colloidal characteristics of the product were immediately destroyed, i.e., separation of 80 cc. of water/95 cc. of finished product. With the purity of the chalk as a factor, precipitated calcium carbonate of the U. S. P. was substituted for the prepared chalk with the result that 60 cc. of water/95 cc. of finished product separated. The *p*H of Magma Bentonite U. S. P. was 8.4 while the *p*H of a freshly prepared 4% silica suspension was 4.95.

DISCUSSION

Calcium carbonate appears to be a positively charged particle while SiO_2 appears to be a negatively charged particle; therefore the chalk mixture sol is coagulated by silicon dioxide. In the Calamine Lotion the calamine is a positively charged sol while the silica is negatively charged sol but no coagulation takes place. This may be due to preferential adsorption of the charges on the surface of particles; more important, however, is the fact that the solubility product of zinc hydroxide is about one-half the solubility product of calcium carbonate. The negative charge of the silicon dioxide would be immediately neutralized by the positive charge of the calcium carbonate in solution causing coagulation and precipitation of calcium silicate.

SUMMARY

1. Four per cent microcrystalline silica appears to be superior to five per cent bentonite magma in Calamine Lotion of the U. S. P.

2. Four per cent microcrystalline silica appears to be inferior to 5 per cent bentonite magma in Chalk Mixture of the U. S. P.

3. Reasons are advanced for the success and failure of this material as a suspending agent.

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The Effects of B Vitamins, Liver, and Yeast on Promin Toxicity in the Rat*

By B. H. ERSHOFF and H. B. McWILLIAMS†

Toxic effects resulting from the administration of massive doses of promin were largely counteracted in rats by the administration of desiccated whole liver. The protective factor was distinct from any of the known vitamins and was not present in the casein or salt mixture employed. The suggestion is mad ethat many of the toxic effects observed in animals fed massive doses of certain drugs are in reality due to a deficiency of an unknown nutrient(s) resulting from an increased requirement following drug administration. Desiccated whole liver is a rich source of this unknown factor(s).

PROMIN (sodium *p,p'*-diaminodiphenylsulfone-N,N'-didextrose sulfonate) has been employed experimentally in the treatment of tuberculosis, leprosy, and infections caused by the hemolytic streptococcus, the pneumococcus, and the gonococcus (1–5). Toxic reactions following the administration of promin have discouraged the use of this drug, particularly with the advent of promizole, a related sulfone, considerably less toxic than promin, and also of value in the treatment of experimental tuberculosis (6, 7). Toxic effects of promin administration may be counteracted, however, to a large extent by dietary

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† We are indebted to Dr. L. A. Sweet of Parke, Davis and Co., Detroit, Mich., for the promin employed in the present experiment.

means. Higgins (8) observed that an increased intake of thiamine, riboflavin, and pyridoxine exerts a protective effect in animals receiving massive doses of this drug. More recently Higgins (9) found that toxic effects might be controlled further by the administration of whole liver or yeast. In the present communication further data are presented on the effects of diet on promin toxicity in the rat.

PROCEDURE AND RESULTS

Four basal rations were employed in the present experiment: diets A, B, C, and D. Diets A and B were purified rations containing the B complex factors in synthetic form only. Diets C and D were similar in composition but contained yeast or desiccated whole liver in addition to the synthetic vitamins. All four rations were supplemented with 0.0 or 5.0 Gm. of promin (sodium *p,p'*-diaminodiphenylsulfone-N,N'-didextrose sulfonate) per Kg. of diet. In addition to the above, two further rations (diets E and F) were employed, similar to diet D but containing the water-soluble extract and the water-insoluble residue of liver, respectively, in place of the whole liver. Eighty-four female rats of the Sprague-Dawley strain were selected at twenty-one to twenty-three days of age and an average weight of 44.2 Gm. for the present experiment. Animals were kept in metal cages with raised screen bottoms to prevent access to feces (two animals per cage); and were fed *ad lib.* the diets listed in Table I. Feeding was continued for eight weeks or until death, whichever occurred sooner. Animals were autopsied on the fifty-sixth day of feeding, and thyroid, ovarian, and adrenal weights determined. Results are summarized in Table II.

Findings confirm the earlier work of Higgins (9) that toxic effects of promin administration may be counteracted in the rat by dietary means. Toxic effects were most pronounced on the synthetic ration (diet A_1) and least evident on the ration containing extracted liver residue (diet F_1). Growth was retarded in all promin-fed rats. Gain in body weight was greater, however, on diets D_1, E_1, and F_1 than on other promin-containing rations. Growth was particularly marked in animals fed the extracted liver residue (diet F_1).[1] On promin-free diets no significant difference in growth was observed on any of the rations employed. The effects of promin administration were most pronounced during the first week of feeding. During this period a considerable number of animals lost weight, particularly those on synthetic rations (diets A_1 and B_1). Forty per cent of the rats on diet A_1 and 30% of those on diet B_1 died at this time. A marked hyperirritability of all promin-fed rats was noted, particularly during the first four days of feeding. Animals were observed chewing each others ears and tail and upper back; and a considerable loss of hair occurred, particularly on the synthetic rations (diets A_1 and B_1). After the first week of feeding growth was resumed in all groups and a marked reduction of irritability was noted

The protective effect of extracted liver residue in promin-fed rats was similarly noted in respect to gonadal weight. Ovarian development was markedly inhibited in promin-fed rats on the synthetic rations (diet A_1 and B_1) and the ration containing yeast (diet C_1). Ovaries were somewhat larger on diets D_1 and E_1 containing whole liver and water-soluble extract of liver, respectively. Ovarian weights were virtually normal, however, in promin-fed rats receiving extracted liver residue (diet F_1). No significant difference in ovarian weight was noted on promin-free diets on any of the rations employed (Table II).

A marked goitrogenic effect was observed following promin administration on all diets employed. Thyroid weights on promin-containing rations averaged 33.5 to 60.7 mg. per 100 Gm. body weight in contrast to values of 5.5 to 6.6 mg. per 100 Gm. body weight on promin-free rations (Table II). No correlation was obtained, however, between the extent of thyroid enlargement and other manifestations of promin toxicity.

A significant difference in adrenal weight was observed in promin-fed rats on the various rations (Table II). The adrenal weight of promin-fed rats receiving extracted liver residue (diet F_1) was significantly greater than that of promin-fed rats on other diets with the possiole exception of diet D_1. When expressed in terms of mg. of adrenal weight per 100 Gm. of body weight, however, no significant difference was noted between the various groups. Adrenal weight in mg. per 100 Gm. of body weight was significantly greater for all promin-fed rats than for animals fed similar rations with promin omitted. In the promin series adrenal weights averaged 37.4 to 39.8 mg. per 100 Gm. of body weight on the various diets employed; in promin-free controls the average range was 24.9 to 28.5 mg. per 100 Gm. of body weight (Table II).

During the eighth week of feeding total and differential white cell counts, hemoglobin determinations, reticulocyte counts and total red cell counts were made on the tail blood of surviving rats on diets A_1, F_1, and A_2. No significant difference in total and differential white cell counts were observed in animals fed any of the above diets. The erythrocyte count and hemoglobin level were somewhat depressed on diet A_1, with erythrocytes averaging 5.8 million (range 4.5 to 6.2) per cubic millimeter of blood and hemoglobin averaging 12.4 Gm. (range 11.4 to 13.7) per 100 cc. of blood. The average erythrocyte count on diet F_1 was 8.1 million (range 7.8 to 8.5) per cubic millimeter of blood, and the average determination of hemoglobin was 15.1 Gm. per 100 cc. of blood (range 14.8 to 15.8). On diet A_2 the average erythrocyte count was 8.0 million (range 7.6 to 8.3) per cubic millimeter of blood, and the average hemoglobin determination was 15.3 Gm. (range 14.5 to 16.3). Reticulocyte counts on the three diets averaged 12.0% on diet A_1 (range 6.9 to 16.0%), 9.9% on diet F_1 (range 4.1 to 17.0%), and 7.0% on diet A_2 (range 4.7 to 11.0%). In previous work Higgins (9) reported a marked reticulocytosis and a significant depression in the erythrocyte count and hemoglobin level of rats fed promin and maintained on a synthetic ration.

[1] Statistically, gain in body weight was significantly greater on diet F_1 than on diet A_1 (see Table II). Furthermore, no overlapping occurred between the two groups, the largest animal on diet A_1 gained less weight than the smallest on diet F_1.

TABLE I.—COMPOSITION OF EXPERIMENTAL DIETS[a]

Components of Basal Mixture	Diets A_1 and A_2	Diets B_1 and B_2	Diets C_1 and C_2	Diets D_1 and D_2	Diet E_1	Diet F_1
Yeast[b]	0.0	0.0	10.0	0.0	0.0	0.0
Whole Liver Powder[c]	0.0	0.0	0.0	10.0	0.0	0.0
Liver Concentrate 1:25[d]	0.0	0.0	0.0	0.0	4.0	0.0
Extracted Liver Residue[e]	0.0	0.0	0.0	0.0	0.0	10.0
Casein[f]	22.0	22.0	22.0	22.0	22.0	22.0
Salt Mixture[g]	4.5	4.5	4.5	4.5	4.5	4.5
Sucrose	73.5	73.5	63.5	63.5	69.5	63.5
Vitamin supplements						
Fat-soluble vitamins: *for all diets*, see footnote [h].						
B vitamins: *for diets* A, C, D, E, and F, see footnote [i]; *for diet* B, see footnote [j].						

[a] Promin (sodium p,p'-diaminodiphenylsulfone-N,N'-didextrose sulfonate) was incorporated in diets A_1, B_1, C_1, D_1, E_1, and F_1 at a level of 5.0 Gm./Kg. of diet, replacing an equal amount of sucrose.
[b] Brewer's Type Yeast No. 200, Anheuser-Busch, Inc., St. Louis, Mo.
[c] Whole Dried Liver Powder, Armour and Co., Chicago, Ill.
[d] Liver Extract Concentrate 1:25, Armour and Co., Chicago, Ill.
[e] Liver Residue Powder, Armour and Co., Chicago, Ill.
[f] Vitamin Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.
[g] Sure's Salt Mixture No. 1 (10).
[h] Each rat received 3 times weekly the following supplement: cottonseed oil (Wesson) 500 mg., alpha-tocopherol acetate 1.5 mg., and a vitamin A–D concentrate containing 50 U. S. P. units of vitamin A and 5 U. S. P. units of vitamin D. The concentrate used was Nopco Fish Oil Concentrate assaying 800,000 U. S. P. units of vitamin A and 80,000 U. S. P. units of vitamin D per Gm.
[i] To each Kg. of diets A, C, D, E, and F were added the following synthetic vitamins: thiamine hydrochloride 72 mg., riboflavin 9 mg., pyrodoxine hydrochloride 15 mg., calcium pantothenate 67.2 mg., nicotinic acid 60 mg., 2-methyl-1,4-naphthoquinone 5 mg. and choline chloride 1.2 Gm. The thiamine, riboflavin, pyridoxine, pantothenate, and nicotinic acid were administered in amounts comparable to that provided by Brewer's Type Yeast No. 200 when fed at a level of 10% of the diet. The amounts fed were considerably in excess of minimum requirements for the rat.
[j] To each Kg. of diet B were added thiamine hydrochloride 144 mg., riboflavin 18 mg., pyridoxine hydrochloride 30 mg., calcium pantothenate 134.4 mg., nicotinic acid 120 mg., p-aminobenzoic acid 600 mg., folic acid 10 mg., biotin 1 mg., 2-methyl 1,4-naphthoquinone 10 mg., choline chloride 1.2 Gm., and inositol 1.2 Gm.

TABLE II.—EFFECTS OF PROMIN ADMINISTRATION ON GROWTH AND THYROID, ADRENAL AND OVARIAN WEIGHT IN THE IMMATURE FEMALE RAT

Dietary Group	Number of Animals	Initial Body Weight, Gm.	Gain in Body Weight Over 8-Week Period, Gm.	Ovarian Weight,[a] Mg.	Adrenal Weight,[a] Mg.	Adrenal Weight, Mg./100 Gm. Body Weight	Thyroid Weight, Mg. Average	Thyroid Weight, Mg. (Range)	Thyroid Weight, Mg./100 Gm. Body Weight
				Promin Series					
A_1	10	44.0	51.2 ± 3.0 (5)[b]	24.8 ± 3.3	36.8 ± 1.4	38.7	35.4	(17.2 – 56.0)	37.3
B_1	10	44.0	45.0 ± 3.8 (7)	21.4 ± 1.8	35.4 ± 2.1	39.8	54.0	(40.6 – 88.6)	60.7
C_1	10	44.1	51.5 ± 3.6 (10)	23.0 ± 2.9	35.5 ± 2.9	37.4	32.2	(20.0 – 40.2)	33.5
D_1	10	44.1	67.8 ± 3.1 (8)	30.8 ± 1.8	44.5 ± 3.3	39.7	50.3	(38.1 – 70.4)	44.9
E_1	10	44.3	64.9 ± 2.6 (9)	31.8 ± 2.8	41.2 ± 1.3	37.8	58.0	(32.0 – 98.6)	53.2
F_1	10	44.4	93.9 ± 2.7 (9)	46.7 ± 2.9	51.6 ± 2.2	37.4	62.1	(34.3 – 114.6)	45.0
				Promin-free Controls					
A_2	6	44.5	135.7 ± 5.8 (6)	52.3 ± 3.2	44.8 ± 1.5	24.9	11.9	(10.0 – 15.1)	6.6
B_2	6	44.3	140.0 ± 3.7 (6)	58.4 ± 2.7	49.7 ± 1.5	27.0	10.5	(9.3 – 12.2)	5.7
C_2	6	44.2	133.4 ± 5.5 (6)	55.7 ± 2.0	49.3 ± 2.4	27.7	10.3	(9.1 – 11.0)	5.8
D_2	6	44.0	152.3 ± 5.1 (6)	57.9 ± 2.8	55.8 ± 2.7	28.5	10.7	(8.0 – 14.2)	5.5

[a] Including standard error of the mean calculated as follows: $\sqrt{\frac{\sum d^2}{n-1}} / \sqrt{n}$ where d is the deviation from the mean and n is the number of observations.
[b] The values in parentheses indicate the number of animals which survived and on which averages are based.

Under conditions of the present experiment, however, the blood picture of promin-fed rats on the synthetic ration (diet A_1) was virtually normal.

DISCUSSION

It is becoming increasingly apparent that the effects obtained in an experimental animal following administration of drugs or related products are dependent on the nutritional state and the diets employed. In acute deficiencies of essential nutrients it is readily recognized that resulting abnormalities in cellular metabolism may profoundly affect response to certain drugs. What is less well recognized, however, is that these drugs themselves

may precipitate a deficiency state. Thyroid administration, for example, may induce a deficiency of thiamine, pyridoxine, and pantothenic acid (11), while dicumarol, salicylates and other drugs may precipitate a deficiency of vitamin K (12–14). In addition to the known nutrients, however, requirements for various unknown factors may also be increased following the administration of certain drugs. These "minor vitamins" are apparently dispensable under normal conditions or their requirements are so small they may readily be met by amounts present in the diet or through the synthetic activity of the intestinal flora or the animals' own tissues. Certain drugs or other "stress factors" may, however, increase requirements for these substances to the extent that deficiencies occur, manifested by retarded growth or tissue pathology, and preventable by the administration in appropriate amounts of the missing nutrient (15). Whole liver is a potent source of such unknown nutrients. The beneficial effects of liver in animals inhaling carbon tetrachloride or fed toxic amounts of strychnine, sulfanilamide, dinitrophenol, atabrine, and other drugs have been recognized for years (16–18). Similar results have been observed following toxic doses of diethylstilbestrol (16), alpha-estradiol (19) and desiccated thyroid (20–22). In the present experiment, toxic effects of promin, as previously reported by Higgins (9), were similarly modified by dietary means.

Findings indicate that the effects of promin administration in the immature female rat were dependent in part on the diet employed. A marked retardation in growth was observed on all rations. Gain in body weight was greater, however, on diets containing liver or liver fractions (diets D_1, E_1, and F_1) than on the synthetic rations (diets A_1 and B_1) or the ration containing yeast (diet C_1). Growth was particularly marked on diet F_1 (containing the water-insoluble extracted liver residue). On promin-free rations no significant difference on growth was observed on any of the diets employed. Similar findings were obtained in respect to ovarian weight. A marked inhibition of ovarian development was observed in promin-fed rats on synthetic rations and the diet containing yeast. Ovarian weight was somewhat greater in promin-fed rats on diets containing whole liver or water-soluble liver concentrate. Ovarian weights were virtually normal, however, on promin-fed rats receiving the water-insoluble extracted liver residue. On promin-free rations no significant difference in ovarian weight was observed on any of the diets employed. Available data indicate that the beneficial effects of liver and liver fractions on growth and ovarian development in the immature promin-fed rat were not due to any of the known B vitamins. This is indicated by the fact that body and gonadal weight of animals fed diet B_1 was significantly less than that of animals fed diets D_1, E_1 and F_1 although diet B_1 contained all known members of the vitamin B complex[3] in amounts exceeding their content in the latter rations. It is suggested, therefore, that whole liver contains some factor(s) other than the known B vitamins whose requirement is increased following prolonged promin feeding, and that this factor(s) is present primarily in the water-insoluble fraction. This factor is not present in significant amounts in yeast.

No measurements of food consumption were made during the experiment, but it was apparent that promin-fed rats on the synthetic rations (diets A_1 and B_1) ate less food than those receiving the liver supplements. This was particularly marked during the first week of feeding. It is not unlikely that the differences in growth and gonadal development of promin-fed rats on the various rations reflect differences in caloric intake. The paired feeding technique might be employed with advantage in such experiments. On the other hand since promin was mixed in the diet, animals with the largest food consumption were also ingesting the largest doses of promin. An increased caloric intake under these conditions might indicate, therefore, that the diet contained some factor(s) which increased resistance to promin intoxication.

In addition to retardation of growth and inhibition of ovarian development, promin administration under conditions of the present experiment resulted in a marked enlargement of the thyroid gland similar to that caused by promizole (23) and other goitrogenic agents (24). Adrenal weight (expressed in mg. per 100 Gm. of body weight) was also significantly increased in all promin-fed rats. These effects occurred on all promin-containing rations and were not inhibited by any of the diets employed.

[3] The recently crystallized vitamin B_{12} is a possible exception to this statement. The latter substance, however, is water-soluble and appears to be distinct from the protective factor in the present experiment which is retained in the water-insoluble fraction of liver.

SUMMARY

The administration of toxic doses of promin to immature female rats maintained on a synthetic diet resulted in a marked retardation of growth and inhibition of ovarian development. These effects were largely counteracted by the administration of a water-insoluble fraction of liver. The protective factor(s) was distinct from any of the known B vitamins and was not present in significant amounts in yeast. The suggestion is made that whole liver contains at least one factor other than the known B vitamins whose requirement is increased in the promin-fed rat.

Other effects of promin administration included a marked increase in thyroid weight and an increase in the ratio of adrenal to body weight. These effects were not counteracted by administration of the liver factor.

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Some Pharmaceutical Applications of Statistical Sampling Plans*

By R. H. NOEL†

Specific illustrations of single, double, and sequential sampling plans for various attribute characteristics are presented. Also included is an illustration of the statistical technique employed in the sampling of substances for which the quality characteristics are expressed as a continuous variable. The use of Analysis of Variance in acceptance sampling is illustrated.

SAMPLING inspection for acceptance purposes is carried out at many stages in pharmaceutical manufacturing. The inspection or control analysis is usually performed on a sampling basis because of the destructive nature of much of the testing employed. For this reason, and because of various economic considerations, one hundred per cent testing is precluded.

The pharmaceutical industry has long recognized this problem and as a result it has been responsible for developing rigidly controlled manufacturing systems which are operated by highly trained production personnel. Although these manufacturing controls provide the largest measure of quality assurance, the control laboratories must perform the necessary inspection to insure the desired quality level. Much of this inspection must be performed on a sampling basis whether the testing involves raw materials, packaging materials, intermediate products or finished products. The purpose of this paper is to illustrate some applications of well-known statistical sampling plans to some of the inspection problems of the industry. No attempt will be made to discuss the statistical theory involved as this has been done adequately elsewhere (1–4).

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I. ATTRIBUTE INSPECTION

Consider first, the problems of attribute inspection which arise during the examination of packaging materials, particularly when such material is to be handled in modern, automatic packaging machines. For example, the examination of collapsible tubes for length, diameter, wall thickness, threads, cap diameter, and smears and scratches on lithograph work; closures for size, thread uniformity, pitch and strength of plastic; cap liners for type and resistance; cartons for position of glue flaps, folding and gluing defects, and printing errors constitute some of the many characteristics which require control in order to obtain efficient packaging output.

It is recognized immediately that defects of the kind mentioned above will ordinarily be detected and eliminated at some subsequent inspection station. However, the efficiency of the packaging operation may be considerably impaired if too much defective packaging material is allowed to reach that stage. How much inspection of such material is necessary is, therefore, determined in the light of economy. The problem is one of choosing an inspection plan which will involve a minimum amount of inspection expense, and simultaneously insure that no more than a specific proportion of defects will reach the next stage.

Consider the examination of collapsible tubes for the multiple defects mentioned above. Without one hundred per cent inspection, it would be impossible to insure that every tube will meet all specifications. One hundred per cent inspection, however, would be in all probability far too expensive. Therefore, it is necessary to agree to accept a certain proportion of defective tubes, which proportion shall be determined in the light of the economy of inspection and processing. For example, it may be economically feasible to use lots of tubes where the average proportion defective for all causes is 1.0%. It may be totally uneconomical to utilize lots where the proportion defective is 2.5%. Since one hundred per cent inspection has been adjudged too expensive, it will be necessary to consider the risks involved in accepting bad lots of tubes and in rejecting good lots

of tubes. When values for these two risks have been assigned, it is possible to construct three types of sampling plans: i.e., single, double, and sequential. Assuming that unsatisfactory quality lots should be rejected 95% of the time and satisfactory quality lots should be accepted 95% of the time, it is possible to construct a single sampling plan as follows:

Let

p_1 = acceptable fraction defective = 0.01
p_2 = unacceptable fraction defective = 0.025
α = risk of rejecting a lot of p_1 quality = 0.05
β = risk of accepting a lot of p_2 quality = 0.05

$$R_0 = p_2/p_1 = \text{operating ratio} = \frac{0.025}{0.01} = 2.5$$

Tables for designing sampling schemes have been published by Peach (1) for single and double sampling. They have been reproduced in Tables I and II Find R_0 in Table I. If it does not appear read the next higher number. Read directly the acceptance number c and sample index np_1. Divide np_1 by p_1 to get n, the sample size. From the table, c = 12 and np_1 = 7.69 for R_0 = 2.5 and np_1/p_1 = 769. The sampling scheme, therefore, calls for a sample size of 769 and an acceptance number of 12. That is, if no more than 12 defects are found in a random sample of 769 tubes, the probability is 0.95 that the lot is less than 2.5% defective and the lot is accepted. If more than 12 defects are found, the lot is rejected. If 769 appears to be a rather large sample, it would be desirable to investigate methods of reducing this number. This could be accomplished by increasing the risks, α and β, but the safer and more effective means would be to investigate the economic effect of increasing p_2.

Assuming the same set of conditions, it is possible to design a double sampling plan as follows:

$p_1 = 0.01$
$p_2 = 0.025$
$\alpha = 0.05$
$\beta = 0.05$

$$R_0 = p_2/p_1 = \frac{0.025}{0.01} = 2.5$$

From Peach's table for double sampling (Table II), read for R_0 = 2.6 (next largest number from 2.5), c_1 = 5, k_1 = 11, c_2 = 13, and n_1p_1 = 4.34. Calculate $n_1 = n_2 = n_1p_1/p_1 = 4.34/0.01 = 434$. Take a random sample of 434 tubes and inspect them for defects. If no more than 5 tubes are defective, accept the lot. If 11 tubes or more are defective, reject the lot. If more than 5 tubes but less than 11 tubes are defective, take another 434 tubes and inspect them. If no more than 13 tubes are defective in the combined samples, accept the lot. If more than 13 tubes are defective in the combined samples, reject the lot. The lot in question, whether passed on the first or second sample, will have a probability of 0.95 of being less than 2.5% defective. Other considerations being equal, the amount of inspection necessary in double sampling is less than for single sampling and is especially useful where the average percentage defective is low. Still another solution is available which requires, on the average, less sampling. This is known as sequential analysis (2).

Using the same numerical quantities as before, the sequential plan is devised as follows:

$p_1 = 0.01$
$p_2 = 0.025$
$\alpha = 0.05$
$\beta = 0.05$

$$g_1 = \log \frac{p_2}{p_1} = \log \frac{0.025}{0.01} = 0.3979$$

$$g_2 = \log \frac{1 - p_1}{1 - p_2} = \log \frac{0.99}{0.975} = 0.0065$$

$$a = \log \frac{1 - \beta}{\alpha} = \log \frac{0.95}{0.05} = 1.2787$$

$$b = \log \frac{1 - \alpha}{\beta} = \log \frac{0.95}{0.05} = 1.2787$$

$$h_1 = \frac{b}{g_1 + g_2} = \frac{1.2787}{0.3979 + 0.0065} = 3.16$$

$$h_2 = \frac{a}{g_1 + g_2} = \frac{1.2787}{0.3979 + 0.0065} = 3.16$$

$$s = \frac{g_2}{g_1 + g_2} = \frac{0.0065}{0.3979 + 0.0065} = 0.0161$$

Two parallel lines may now be computed according to the equations: $d_2 = h_2 + sn$ for the upper line and $d_1 = -h_1 + sn$ for the lower line, where n is equal to the number of items being inspected. The lines are plotted on cross-hatched paper as shown in Fig. 1. The vertical axis represents the total number of defectives found and the horizontal axis represents the total number of tubes examined.

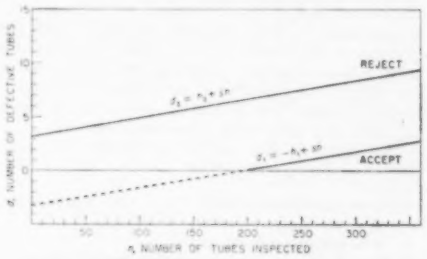

Fig. 1.—Sequential Plan—Graphical Solution Key: p_1 = .01; p_2 = .025; α = .05; β = .05.

The inspection consists of examining one tube at a time drawn at random from the lot. After each tube is inspected, the inspector plots the total number of defective tubes against the total number of items examined. Each plotted point is thus always one space to the right of the preceding point and it is either on the same level or one space higher depending on whether or not a defective tube is found. Inspection continues until a plotted point falls either into the accept or reject area.

The sequential method permits a decision concerning a lot in accord with the predetermined risks and quality levels with less inspection than single or double sampling, other considerations being equal. It is exceptionally efficient when lots of material are excessively bad or exceptionally good, as little inspection is necessary in such cases either to reject or accept.

The respective values of p_1, p_2, α, and β may differ widely with the material to be inspected, economy involved, and the quality level desired.

II. VARIABLES INSPECTION

Much of the inspection associated with raw materials, in process and finished product control is based upon chemical, physical, and biological analysis. With such types of testing procedures, the quantities being measured are usually capable of being represented on a continuous scale. One hundred per cent inspection is hardly ever possible because of the destructive nature of many of the testing procedures.

A simple example of this type of inspection problem would be the routine laboratory check of the label potency of a finished product. Given a lot of vials with a label claim of 5000 units and minimum and maximum average tolerances of 4000 and 6000 units, respectively, how many vials should be tested in order to be reasonably assured that the potency of the product is satisfactory?

To answer this question, some knowledge of the variability between vials in the same lot must be obtained. This variability is usually accounted for largely by the variability of the filling equipment and the error of the analytical technique, since such vials are filled from homogeneous bulk material. Therefore, the average variability between vials of the same lot is or should be known and well controlled. It should be assumed that the standard deviation is known to be approximately 450 units.

Because of the destructive nature of the testing procedure, the laboratory must resort to sampling inspection and it will, therefore, be necessary to assign values to the two risks associated with the decision to be based on the results of the sample tests. As in attribute sampling, these risks are α, the chances of rejecting a lot when it is of satisfactory potency and β, the chances of accepting a lot when it is of unsatisfactory potency.

The sampling plan can be calculated using Clifford's terminology (5). Let

n = number of individuals in the sample
σ = standard deviation = 450
$\bar{x}$ = average of the sample
$\bar{x}_1$ = lower acceptance average for sample
$\bar{x}_2$ = upper acceptance average for sample
u = computed average of particular lot
u_0 = desired value of lot average = 5000
u_1 = lower tolerance value for lot average = 4000
u_2 = upper tolerance value for lot average = 6000
α = risk of rejecting lot if $u = u_0 = 0.05$
β = risk of accepting lot if $u = u_1$ or $u_2 = 0.05$
$t(\alpha)$ = the value of t on a normal curve corresponding to a one-tail area of α
$t(\beta)$ = the value of t on a normal curve corresponding to a one-tail area of β

Calculate:

$$n = \sigma^2[t(\alpha/2) + t(\beta)]^2/(u_0 - u_1)^2$$
$$n = 450^2[1.960 + 1.645]^2/(5000 - 4000)^2$$
$$n = 2.63$$
$$\bar{x}_1 = t(\alpha/2)u_1 + t(\beta)u_0/[t(\alpha/2) + t(\beta)]$$
$$\bar{x}_1 = [(1.960)(4000) + (1.645)(5000)]/[1.960 + 1.645] = 4456$$

Since $\bar{x}_2$ will be as much above u_0 as $\bar{x}_1$ is below it, we find $\bar{x}_2 = 5544$. From the above calculations, our sampling plan is defined as follows:

1. Analyze 3 vials separately.
2. Calculate the average of the sample $\bar{x}$.
3. If $\bar{x}$ is between 4456 and 5544, accept the lot.
4. If $\bar{x}$ is less than 4456 or over 5544, reject.

III. APPLICATION OF VARIANCE ANALYSIS TO A CHEMICAL SAMPLING PROBLEM

It is not uncommon for the purchasing agent or the production manager to request an estimate of the active constituent in a large shipment of raw material. The purchasing agent is interested in learning whether he has purchased what he has specified and, moreover, he is interested in a rapid answer in order that he may take advantage of discounts on cash payments. The production manager may be interested from many standpoints, not the least of which is that he must be reasonably sure that sufficient active constituent is available to produce a specific amount of finished product over a period of time.

Assuming that adequate analytical methods are available for the precise determinations of the active constituent involved, one must concentrate upon obtaining a sample which is representative of the shipment. That the content of the active constituent in raw materials may vary from time to time, is unquestioned. What is more disconcerting is the fact that the variability between shipments, lots, barrels, bags, etc., may be such that no standard sampling technique will serve for every instance. It, therefore, becomes important to sample in such a manner that a measure of all possible variation will be obtained. What is more important is to be able to separate the total variability inherent in the shipment into the component parts which are associated with each factor such as lots, barrels, bags, etc. Once this information is available, it is quite possible to define the exact method of taking one additional sample which will supply all the information necessary to make a precise estimate of the quality of the shipment.

The answer to some of these problems can be found by application of a statistical technique known as "Variance Analysis," the fundamentals of which are described in many standard texts (6–8). The following example will serve to illustrate such an application:

One consignment of concentrated aluminum hydroxide gel was received in two lots of 50 barrels each. The consignment was alleged to be homogeneous. The label claim was 8% plus or minus 1% Al_2O_3.

The production department wished to know if the true mean value of the entire consignment was between 7% and 9% Al_2O_3 so that they could determine whether or not they would have enough material to process a desired amount of product containing 4% Al_2O_3. Further, it was the responsibility of the control department to accept or reject the material on the basis of 8% plus or minus 1% Al_2O_3 as per purchasing specifications.

The nature of this material was such that at first glance it would seem that every barrel would require

an analysis, for water had separated and some settling had occurred. For the purpose of acceptance sampling, however, such detailed sampling was not necessary, as will be shown below.

It was decided to take two samples from each of two barrels from each lot and perform duplicate analyses. It is obvious that the purpose of this sampling was to ascertain the variability between lots, between barrels, within lots, between samples within barrels as compared with the variability in the analytical technique.

The results are tabulated below:

Lot 1, % Al_2O_3

	Barrel 1		Barrel 2	
Assay	Sample 1	Sample 2	Sample 1	Sample 2
1	8.2	8.9	7.2	9.1
2	8.1	9.0	7.3	9.0

Lot 2, %

	Barrel 1		Barrel 2	
Assay	Sample 1	Sample 2	Sample 1	Sample 2
1	10.0	8.9	10.0	9.0
2	10.1	9.2	10.3	9.2

Analysis

1. Square each individual value, and sum these squares, i.e.,

$$(8.2)^2 + (8.1)^2 \ldots + (9.2)^2 = 1299.59$$

2. Square each sample block total, sum these squares and divide by the number of individuals making up each total, i.e.,

$$\frac{(8.2 + 8.1)^2 + \ldots + (9.0 + 9.2)^2}{2} = 1299.46$$

3. Square each barrel block total, sum these squares and divide by the number of individuals making up each total, i.e.,

$$\frac{(8.2 + 8.1 + 8.9 + 9.0)^2 + \ldots + (10.0 + 10.3 + 9.0 + 9.2)^2}{4} = 1293.47$$

4. Square each lot block total, sum these squares and divide by the number of individuals making up each total, i.e.,

$$\frac{(8.2 + 8.1 + \ldots + 9.0)^2 + (10.0 + 10.1 + \ldots + 9.2)^2}{8} = 1293.14$$

5. Obtain the grand total of all observations, square this total and divide by the total number of individual items, i.e.,

$$\frac{(8.2 + 8.1 + \ldots + 9.2)^2}{16} = 1287.02$$

The Variance Analysis Table is set up as indicated in Table III.

The sums of squares for the Variance Analysis Table are obtained as is outlined at the bottom of this page.

It is of first importance to learn whether the between samples variability is sufficiently large to be detected by the analytical method employed. In this case, the mean square value for the samples is 1.4975 and that for the duplicate analyses is 0.01625. It is easily seen that the between sample means square is 92.15 times as large as the between duplicate analysis mean square (1.4975/0.01625). It now becomes a matter of determining if the magnitude of this ratio (92.15) can be considered statistically significant. This ratio is known as the F ratio and probability tables for determining the significance of F are available (7). It was determined from such a table (4 and 8 degrees of freedom) that the analytical method would only fail 5 times in 100 if the ratio were 3.84 and only one time in 100 if the ratio were 7.01 to detect the large variation associated with samples from the same barrel. Since the calculated value far exceeds either tabular value, it is concluded that the variability between samples within a barrel is real and can be detected.

If the variation between barrels is not as great as that within barrels, one cannot detect such a variation from a multiple analysis of the barrel contents. Attention is now focused on the between barrel mean square. The ratio of this mean square to the mean square for samples is obtained: 0.165/1.4975 = 0.110. The tabular value for F at p = 0.05 with 2 and 4 degrees of freedom is 6.94. Inasmuch as the calculated ratio is far less than the tabular value, it should be concluded that variability between barrels is not significant and, therefore, does not exist.

To determine whether a significant variation exists between lots, the mean square for lots is tested against the pooled mean square for samples and barrels, viz.:

$$0.33 + 5.99 = 6.32$$
$$6.32/(2 + 4) = 1.0533$$
$$F = 6.12/1.0533 = 5.81$$

From the F table, the value 5.99 is found for 1 and 6 degrees of freedom at P = 0.05. Since the calculated value is so close to the tabular value, it is suspected that the variation between lots is real and can be detected.

From the equations listed in the column headed "Components of Variance," the following are calculated:

$$(s_4)^2 = 0.0162$$
$$(s_3)^2 = \frac{1.49750 - 0.0162}{2} = 0.7406$$
$$(s_2)^2 = 0 \text{ as determined above}$$
$$(s_1)^2 = \frac{6.12 - 1.4975}{8} = 0.5778$$

The total variance of a single analysis can be calculated as: $(s_1)^2 + (s_2)^2 + (s_3)^2 + (s_4)^2 = 0.5778 + 0.0 + 0.7406 + 0.0162 = 1.3346$.

The standard deviation is the square root of the total variance: $s = \sqrt{1.3346} = 1.16\%$.

Between lots	(4) − (5)	1293.14 − 1287.02 =	6.12
Between barrels within lots	(3) − (4)	1293.47 − 1293.14 =	0.33
Between samples within barrels	(2) − (3)	1299.46 − 1293.47 =	5.99
Between duplicate analyses	(1) − (2)	1299.59 − 1299.46 =	0.13
Total sum of squares	(1) − (5)	1299.59 − 1287.02 =	12.57

Thus for a single analysis on one sample from one barrel from one lot, the estimate of the Al_2O_3 content with an adequate degree of certainty would only be within $\pm 1.96 \times 1.16 = 2.27\%$ of the true value (6, 7). It is obvious that some other sampling technique must be employed since the estimate must be within $\pm 1\%$ of the true value.

Concentrating attention on the factors contributing the greatest variance, a sampling procedure can be devised to reduce this error of estimate to the required level.

Since (s_2) does not exist, the number of barrels to be sampled is not important because the analysis has indicated that the barrels are similar even though their contents are not homogeneously mixed.

Increasing the number of samples from any one barrel would decrease the error of estimate only as affected by within-barrel heterogeneity.

Increasing the number of barrels sampled from each lot would decrease the error of estimate only as affected by lot variability.

The conclusion is that there is a difference in the Al_2O_3 content of the two lots. The lots are in themselves homogeneous; the variation within barrels of the same lot arises from the settling which occurred on standing. Therefore, either 1 sample from each of 10 barrels in each lot or 10 samples from 1 barrel in each lot will equally increase the precision of the estimate of the lot difference. Using the latter scheme, however, will simultaneously provide an increase in the precision of the estimate of within barrel differences. The number of analyses would not necessarily increase because blending of the samples has the effect of adding the variances into a homogeneous sample.

Therefore, it was decided to observe the effect of taking 10 samples with a sampling thief from 1 barrel from each lot, and blending all samples. The estimated variance of an analysis performed on the blended sample would be:

$$\frac{0.5778 + 0.7406}{10} + 0.0162 = 0.1480$$

and the standard deviation:

$$s = \sqrt{0.1480} = 0.385\%$$

The estimated Al_2O_3 content would now be within $\pm 1.96 \times 0.385 = 0.755\%$ which is adequate for the problem at hand. Thus, with one more sample taken as described above and with but a single additional analysis, the Al_2O_3 content of the shipment can be estimated well within the desired range of $\pm 1\%$ Al_2O_3.

SUMMARY

The use of some statistical sampling methods in pharmaceutical inspection problems has been

TABLE I.—FOR DESIGNING SINGLE SAMPLING SCHEMES ($\alpha = \beta = 0.05$)[a]

R_0	c	n_1p_1
58.	0	0.051
13.	1	0.355
7.5	2	0.818
5.7	3	1.366
4.6	4	1.970
4.0	5	2.61
3.6	6	3.29
3.3	7	3.98
3.1	8	4.70
2.9	9	5.43
2.7	10	6.17
2.5	12	7.69
2.37	14	9.25
2.03	21	14.89
1.81	30	22.44
1.61	47	37.20
1.51	63	51.43
1.335	129	111.83
1.251	215	192.41

[a] This table was reproduced from Paul Peach's "Industrial Statistics and Quality Control" (1) by permission of the author.

TABLE II.—FOR DESIGNING DOUBLE SAMPLING PLANS ($\alpha = \beta = 0.05$)[a]

R_0	c_1	k_1	c_2	n_1p_1
15.1	0	1	1	0.207
8.3	0	2	2	0.427
5.1	1	3	4	1.00
4.1	2	4	6	1.63
3.5	2	5	7	1.99
3.0	3	7	9	2.77
2.6	5	11	13	4.34
2.3	6	13	16	5.51
2.02	9	17	23	8.38
1.82	13	23	32	12.19
1.61	21	34	50	20.04
1.505	30	45	69	28.53
1.336	63	83	138	60.31

[a] This table was reproduced from Paul Peach's "Industrial Statistics and Quality Control" (1) by permission of the author.

TABLE III

Source of Variation	Degrees Freedom[a]	Sum of Squares	Mean Square	Components of Variance
Between lots	1	6.12	6.12	$n_2n_3n_4(s_1)^2 + n_3n_4(s_2)^2 + n_4(s_3)^2 + (s_4)^2$
Between barrels within lots	2	0.33	0.165	$n_3n_4(s_2)^2 + n_4(s_3)^2 + (s_4)^2$
Between samples within barrels	4	5.99	1.4975	$n_4(s_3)^2 + (s_4)^2$
Between analyses on same sample	8	0.13	0.0162	$(s_4)^2$
Total	15	12.57		

[a] The degrees of freedom are obtained as follows: Let n_1 = number of lots = 2; n_2 = number of barrels from each lot = 2; n_3 = number of samples from each barrel = 2; and n_4 = number of analyses on each sample = 2.

Then the degrees of freedom for: Lots = $(n_1 - 1) = (2 - 1) = 1$; barrels = $n_1(n_2 - 1) = 2(2 - 1) = 2$; samples = $n_1n_2(n_3 - 1) = (2)(2)(2 - 1) = 4$; analyses = $n_1n_2n_3(n_4 - 1) = (2)(2)(2)(2 - 1) = 8$; total = $(n_1n_2n_3n_4) - 1 = (2)(2)(2)(2) - 1 = 15$.

illustrated. Single, double, and sequential sampling methods were applied to the attribute inspection of packaging material. A sampling method for variables inspection of a finished product is included along with an illustration of the use of variance analysis in chemical sampling.

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Studies in the Hydrophilic Properties of Possible Ointment Base Constituents. II. The *n*-Alkyl Alcohols with a Carbon Chain Length of C_{20} to C_{30}*

By ALFRED HALPERN† and WILLIAM J. WILKINS‡

The *n*-alkyl alcohols from C_{20} to C_{30} were synthesized and the physical constants of the alcohols and their intermediates were determined. The water numbers of these alcohols were determined and the optimum concentration of the alcohol causing the maximum water potentiation was noted. The C_{30} alcohol caused the greatest water potentiation and the C_{24} alcohol had the lowest optimum concentration. The alcohols with the carbon chain length greater than 24 had the same optimum concentration, 3 per cent. The study indicates that molecular size and the orientation of the alkyl group (lipophilic) plays an important part in the emulsifying properties of the fatty alkyl alcohols.

WATER numbers of the fatty alcohols with a carbon chain length of C_{10} to C_{18} have been reported (1). The optimum concentration of the fatty alcohols necessary to cause the maximum potentiation of the water absorbing properties of petrolatum increased with the increase in carbon chain length. Cetyl alcohol, however, had the lowest optimum concentration (4%) while stearyl alcohol caused the greatest water absorbtion with 43.2 cc. of water per 100 Gm. of fatty alcohol-petrolatum mixture. Rupp (2) also reported better water absorbing properties of fatty alcohols, with increased carbon chain length.

Since the amount of water absorbed by the fatty alcohol-petrolatum mixtures previously reported (1) continued to increase with the carbon chain length it was of interest to determine the optimum carbon chain length that caused the maximum water absorption of petrolatum. The present study concerns itself with the *n*-alkyl primary alcohols with a carbon chain length from C_{20} to C_{30}.

The higher *n*-alkyl alcohols are present as constituents of natural waxes (3). The isolation of alcohols from these sources was not a convenient procedure. There are many synthetic methods described in the literature for the preparation of these alcohols. They may be prepared by the reduction of the appropriate fatty acid ester. Generally these are prepared by the successive increments of one or two carbon atoms starting from stearic acid (4). Robinson (5) reported a method whereby long chain acids were obtained through one operation. The alcohols could be obtained from the acid through the Bouveault-Blanc reduction of the ester.

Recently, Jones (6) reported a method for the preparation of long chain primary alcohols through the reaction between alkyl zinc compounds with ω-acetoxy acyl halides. This method eliminates the necessity of a Bouveault-Blanc reduction.

$$\underset{\text{(I)}}{RZnX} + \underset{\text{(II)}}{XCO(CH_2)_nCH_2OOCCH_3} \longrightarrow \underset{\text{(III)}}{R{-}CO{-}(CH_2)_nCH_2OOCH_3}$$

$$\text{III} \xrightarrow[HCl]{Zn\text{-}Hg} \underset{\text{(IV)}}{RCH_2 - (CH_2)_n - CH_2OOCCH_3}$$

$$\text{IV} \xrightarrow{NaOH} \underset{\text{(V)}}{RCH_2 - (CH_2)_n - CH_2OH}$$

A limitation of this method was the difficulty in obtaining the hydroxy acids, since these acids

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were obtained from the decomposition of the appropriate ozonide (13).

The alcohols used in this study were prepared through a modification of a method described by Jones (6). Another preparative procedure was employed to obtain eicosyl alcohol.

EXPERIMENTAL

Preparation of Eicosyl Alcohol ($C_{20}H_{41}OH$).—The Grignard reagent was prepared in the usual manner from 0.1 mole (30.4 Gm.) of stearyl chloride and 0.12 mole (2.5 Gm.) of magnesium and 500 cc. of anhydrous ether. When all of the stearyl chloride was added the mixture was stirred for 1 hr. and then a solution of 0.2 mole of ethylene oxide in 300 cc. of anhydrous ether was added dropwise as fast as the condenser would allow. The reaction was very violent. The temperature of the reaction mixture was kept at 0° by means of an ice-salt bath. When the addition was completed the mixture was allowed to warm at room temperature and then worked up in the usual manner. The ether layer was separated, dried over anhydrous sodium sulfate and then the solvent was distilled. The residue was crystallized from benzene, m. p. 69–70°. Yield: 71% theoretical.

An attempt was made at the preparation of docosyl-alcohol in a manner similar to that described above. However, the yield of cosyl bromide prepared by the method described (7) for the preparation of alkyl bromides was 18%. Since a moderately large quantity of the alcohol was needed, this method (i.e., through the Grignard) of obtaining the alcohol was not satisfactory. The alcohol was prepared in a fairly good yield by a method described by Jones (6) employing ethyl succinyl chloride and stearyl zinc chloride. The resulting long chain ketonic acid ester was reduced with amalgamated zinc through the Clemmensen method, to ethyl docosanate. The resulting long chain aliphatic ester was reduced to docosanol through a Bouveault-Blanc reduction.

$$\underset{\text{(I)}}{R{-}ZnX} + X\overset{\overset{O}{\|}}{C}{-}\underset{\text{(II)}}{(CH_2)_n}COOEt \longrightarrow \underset{\text{(III)}}{RCO{-}(CH_2)_n{-}COOEt}$$

$$\text{III} \xrightarrow[\text{Reduction}]{\text{Clemmensen}} \underset{\text{(IV)}}{RCH_2{-}(CH_2)_n{-}COOEt}$$

$$\text{IV} \xrightarrow[\text{Alcohol}]{Na} \underset{\text{(V)}}{RCH_2(CH_2)_nCH_2OH}$$

In a similar manner, the alcohols from C_{24} to C_{30} were prepared.

Preparation of Monoethyl Esters of Dicarboxylic Acids.—The monoethyl esters of the dicarboxy aliphatic acid were prepared through the method described in "Organic Syntheses" (8) for the preparation of ethyl hydrogen sebacate (Table I).

These acid esters were then converted to the acyl chloride by refluxing the appropriate compound with 0.2 *M* excess of thionyl chloride, for four hours. The excess thionyl chloride was removed by distillation under diminished pressure and the acyl chloride distilled at 1–2 mm. pressure.

Preparation of Ketonic Acids.—Stearyl bromide[1] was prepared from Stearyl alcohol[2] in the manner that was described for the preparation of dodecyl bromide (9).

TABLE I.—THE MONOETHYL ESTERS OF THE DICARBOXYLIC ACIDS—$(C_2H_5OOC(CH_2)_nCOOH)$

n =	Formula	B. P., 1–2 Mm.	Yield, %
2	$C_8H_{12}O_4$	139–140	87
4	$C_8H_{14}O_4$	149–150	84
6	$C_{10}H_{18}O_4$	153–154	76
8	$C_{12}H_{22}O_4$	158–160	71
10	$C_{14}H_{26}O_4$	180–181	72

A solution of 0.2 *M* Grignard reagent prepared in the usual way from stearyl bromide, magnesium, and ether, was added to 0.2 mole of zinc chloride (fused and powdered) and 250 cc. of ether, in a 3-necked flask (fitted with a stirrer, reflux condenser and dropping funnel). When all of the Grignard reagent had been added, the mixture was refluxed for two hours and then the ether distilled until the volume was reduced to one half. A solution of 0.2 mole of the appropriate acyl chloride in 200 cc. of benzene was added. The mixture was refluxed for three hours and then hydrolyzed with dilute acid. The layers were separated and the aqueous layer extracted with benzene. The extracts were combined with the nonaqueous layer and the whole washed with acidulated water. The benzene was removed by distillation and the residue hydrolyzed with 250 cc. of 20% NaOH. The acids were regenerated from the sodium salt by acidification with dilute HCl. The crude acid was collected at the pump and air dried. The acids were purified by repeated reprecipitation from alkaline solution (Table II).

The ketonic acids were reduced to the long chain aliphatic acid through a Clemmensen method. The directions given by R. G. Jones (6) for the reduction of 13-ketohentriacontanoic acid and the esterification of the resultant acid to ethyl hentriacontanoate were followed exactly in order to prepare the ethyl esters of the aliphatic acids from C_{22} to C_{30} (see Table III).

A mixed melting point between the docosyl alcohol and an authentic sample[2] showed no depression. Similarly there was no depression of the melting point of an authentic sample of hexacosyl alcohol (ceryl alcohol, E. K.) mixed with the one prepared above.

Melissyl alcohol was reported (10) to be present as the palmitate in beeswax. An attempt was made to isolate this alcohol from this source.

Isolation of "Triacontanol" from Beeswax.—One hundred grams of beeswax (U. S. P.) were refluxed with 300 cc. of 50% alcoholic KOH for eight hours.

[1] A sample of Stearyl Bromide was furnished by the Columbia Chemicals Co., Columbia, S. C., through the courtesy of Mr. Max Gergel.

[2] Furnished by the E. I. Du Pont de Nemours Co., Wilmington, Del., through the courtesy of Dr. J. H. Shipp.

TABLE II.—THE KETONIC ACIDS—CH_3—$(CH_2)_{16}$—CH_2—CO—$(CH_2)_n$COOH

n =	Formula	M. P.°	Yield, %	Neutralization Equivalent[a] Calculated	Found
2	$C_{22}H_{42}O_3$	91–93	78	352.6	350.4
4	$C_{24}H_{46}O_3$	94–96	76	392.3	390.4
6	$C_{26}H_{50}O_3$	97–98	74	410.6	410.2
8	$C_{28}H_{54}O_3$	99–100	68	438.7	437.8
10	$C_{30}H_{58}O_3$	101–102	73	466.7	466.3

[a] Shiner, R. L., and Fusion, R. C., "Systematic Identification of Organic Compounds," J. Wiley and Sons, New York, N. Y., ed. 2, p. 116.

TABLE III.—ETHYL ESTERS OF n-ALKYL ACIDS—$(CH_3—(CH_2)_nCOOEt)$

n =	Formula	M. P. °	Yield, %
20	$C_{24}H_{48}O_2$	45–46	78
22	$C_{26}H_{52}O_2$	49–50	76
24	$C_{28}H_{56}O_2$	53–54	72
26	$C_{30}H_{60}O_2$	58–59	74
28	$C_{32}H_{64}O_2$	63–64	75

The mixture was poured into 2 L. of cracked ice and the flocculent precipitate collected on a Buchner funnel. The precipitate was washed by repeated suspension in 1 L. of warm water, until the supernatant liquid was neutral to litmus (7 washings). The precipitate was collected and dried. The melting point of the precipitated material indicated a mixture of compounds (m. p. 76–90°). Yield: 48 Gm.

A small portion of this precipitate was sublimed at 1–2 mm. pressure. The sublimate was removed periodically. The first portion melted at 76–82°; sublimation point 150–170°; second portion subliming at 185–200°; melted at 84–88°. The fraction subliming at 200–225° melted at 86–94°.

A larger sample (25 Gm.) was sublimed at 185–200° and 1–2 mm. pressure, the middle fraction collected. M. p. 82–86° (17 Gm.).

The melissyl alcohol ($C_{30}H_{61}OH$) prepared synthetically depressed the melting point of the presumed melissyl alcohol isolated from the beeswax. It was probably a mixture of the C_{28} to the C_{32} alcohols. This was in accord with the report (3) that the alcohols isolated from the natural sources and referred to in the literature as ceryl alcohol ($C_{26}H_{53}OH$) and melissyl alcohol ($C_{30}H_{61}OH$) are invariably mixtures of the even numbered homologs. (See Table IV.)

The water numbers of the alcohols were determined in the manner described by Halpern and Zopf (1). To check these values the amount of water in 2 Gm. of the saturated base was then determined by the U. S. P. XIII toluene distillation method (Table V).

The optimum concentrations of the alcohols and the amount of potentiation of hydrophilic properties of petrolatum were correlated to the carbon chain length (Figs. 1 and 2). The values for the series from C_{16} to C_{18} were obtained from the previously reported study (1) by Halpern and Zopf in order to complete the series.

DISCUSSION

The results of the study indicated that the maximum increase of the water number of petrolatum was caused by the C_{20} alcohol. The optimum concentrations of the n-alkyl alcohols decreased with the increase in the carbon chain length. The optimum as reported for cetyl alcohol by Halpern and Zopf (1) indicates that some factor in addition to the chain length or molecular size was a determinant of the optimum concentration of the alcohols. It is important to note that the cetyl alcohol used in their study was labeled cosmetic

TABLE IV.—THE n-ALKYL ALCOHOLS—$CH_3(CH_2)_nCH_2OH$

n =	Formula	Chemical Name	Common Name	Melting Point Recorded	Found
18	$C_{20}H_{41}OH$	Eicosanol	...	71	69–70
20	$C_{22}H_{45}OH$	Docosanol	...	...	71–72
22	$C_{24}H_{49}OH$	Tetracosanol	...	...	74–76
24	$C_{26}H_{53}OH$	Hexacosanol	Ceryl Alcohol	79.5	77–78
26	$C_{28}H_{57}OH$	Octacosanol	...	83.4	82–83
28	$C_{30}H_{61}OH$	Triacontanol	Melissyl Alcohol	86.5	85–86

TABLE V.—WATER NUMBERS[a] OF ALCOHOLS $C_nH(2_n + 1)OH$

n =	Per Cent Concentration in White Petrolatum[b] 1	2	3	4	5	6	7	8	9	10
20	30.3	38.1	43.1	51.2	54.3	48.3	41.0	38.2	36.0	30.0
22	25.1	31.3	40.1	46.3	43.0	38.0	35.0	32.0	30.0	...
24	24.4	30.0	38.0	35.0	34.0	30.4	28.2	27.1	26.4	...
26	23.0	28.0	36.0	35.6	33.0	28.0	26.1	24.3	22.0	20.0
28	26.0	33.0	35.0	34.1	32.4	30.1	28.0	26.0	24.0	23.0
30	21.0	27.0	33.6	33.1	31.6	29.2	27.1	25.0	24.1	20.8

[a] Based on 100 Gm. of the mixture.
[b] The water number of the petrolatum used was 9.5.

grade and it was not known whether it was derived from natural or synthetic sources. The possibility of impurities should not be discounted.

It is difficult to provide an explanation for the apparent nonconformance of cetyl alcohol. Further work is now in progress to ascertain the manner through which possible impurities influence the optimum concentrations of the alcohol and its water number. Casparis and Meyer (11) also reported the optimum concentration of cetyl alcohol to be 4%.

The leveling off of the optimum concentrations with the C_{24} alcohol indicated the possibility of an optimum molecular size for orientation at the water-oil interface. A study of the relationship between the geometric orientation of the fatty alkyl group and emulsifier efficiency has been started and will be reported at a later date.

The amount of water that could be emulsified in petrolatum compared to the carbon chain length had its maxima at the C_{20} alcohol. It is important to note that while there was a decrease in the amount of water that was emulsified in the petrolatum by the alcohols greater than eicosanol, the series with the chain length from C_{20} to C_{30} caused a greater emulsification than the corresponding series from C_{10} to C_{20}. It was shown that hydrogen bonding or association was a factor in explaining the activity of fatty alcohols as emulsifying agents (12). Assuming this property of the hydroxyl group to be a constant for the series studied, then any differences noted would be due to the change in the lipophilic group. Since this change consisted of an increase in molecular size, a large measure of the properties noted of this series of alcohols presumably were determined by the geometric orientation of the lipophile about the water droplet. The *n*-alkyl alcohols greater than C_{10} are all miscible with petrolatum (within the concentration limits of this study), thus the solubility of the alkyl group in the fat phase ceases to become a deciding factor, again pointing to the spacial configuration of the chain as a determinant.

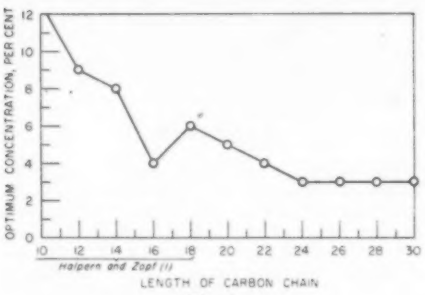

Fig. 1.

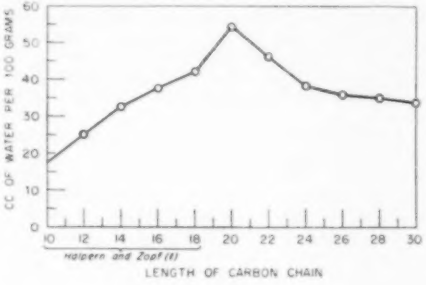

Fig. 2.

SUMMARY

1. The *n*-alkyl alcohols from C_{20} to C_{30} were synthesized and their physical constants and intermediates were determined.

2. The water numbers of these alcohols were determined and the optimum concentration of the alcohol causing the maximum water potentiation was noted.

3. The C_{20} alcohol caused the greatest water potentiation and the C_{24} alcohol had the lowest optimum concentration. The alcohols with the carbon chain length greater than 24 had the same optimum concentration, 3 per cent.

4. The study indicates that molecular size and spacial orientation of the alkyl group (lipophilic) plays an important part in the emulsifying properties of the fatty alkyl alcohols.

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Studies in the Hydrophilic Properties of Possible Ointment Base Constituents. III. The Fatty Mercaptans*

By ALFRED HALPERN† and ARTHUR C. GLASSER‡

The mercaptans with a carbon chain length from C_{10} to C_{18} were synthesized. The physical properties of these compounds and their intermediates were noted. The water numbers of the mercaptans were determined and a correlation was made between the mercaptan series and their hydroxy-analogs. The mercaptan series as a whole showed little ability to emulsify water in petrolatum. The range in water numbers for the mercaptan series was from 15 to 18 at the optimum concentration. The optimum concentration of the mercaptans fluctuated slightly (from 2 to 4 per cent).

THE CHEMISTRY of the mercaptans has been extensively reviewed by Malisoff, Marks, and Hess (1). One outstanding difference between the mercaptan group and the hydroxyl group is the rather limited tendency of the sulfhydryl grouping to undergo association. This has been reported as a result of spectroscopic studies of thiophenol (2) and from heats of mixing studies (3) which showed that the weak donor ability of sulfur was responsible for the failure of thiophenol to be associated. It was also shown (3) that the hydrogen of *n*-heptyl mercaptan did not form bonds with donor oxygen or nitrogen.

The greater association of the lower alcohols causes these compounds to have a higher boiling point than their corresponding sulfur derivatives. In the case of the *n*-heptyl alcohol, its association is enough to compensate for the increased molecular weight of the *n*-heptyl mercaptan so that the boiling point of both these compounds is the same.

As a phase of a study of the mechanisms of hydrophilic ointment formation, it was deemed important to investigate the role of association of the fatty alcohols in these bases. A determination of the water numbers of a homologous series of mercaptans when compared with their hydroxy analogs should give such information. The water numbers of a series of fatty alcohols from C_{10} to C_{18} have been reported (4) and the present study was concerned with the water numbers of a corresponding series of alkyl mercaptans.

Mercaptans have been prepared by the hydrolysis of S-alkylisothiouronium salts (5); the reaction between alkyl halides and metal hydrosulfides (6); hydrolysis of thioesters (7); the addition of hydrogen sulfide to olefins (8); catalytic alkylation of hydrogen sulfide (9); and the acid and alkali reduction of disulfides (10).

The mercaptans[1] used in this study were prepared through the reaction between the alkyl bromide and sodium hydrosulfide in absolute ethanol (I).

$$RX + NaHS \rightarrow RSH + NaBr \qquad (I)$$

The formation of the di-alkyl sulfide was observed to some extent. Apparently this occurred as a result of the sodium mercaptide forming through the reaction between the sodium hydrosulfide and the mercaptan (II).

$$RSH + NaHS \rightleftharpoons RSNa + H_2S \qquad (II)$$

The sodium mercaptide then reacted with another molecule of the *n*-alkyl bromide to form the dialkyl sulfide (III).

$$RSNa + RX \rightarrow RSR + NaX \qquad (III)$$

By increasing the concentration of hydrogen sulfide during the preparation of the sodium hydrosulfide, *in situ*, the formation of the dialkyl sulfide was markedly reduced from 12–19% to 4–6%.

The mercaptans were also prepared through the alkaline hydrolysis of the appropriate S-alkylisothiouronium salt. Dialkylsulfide formation resulted to some extent with this method, too.

The mercaptans were rather easily oxidized by exposure to air and also had a tendency to split out hydrogen sulfide. The mercaptans were converted to the lead mercaptides. The mer-

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[1] The authors gratefully acknowledge the cooperation of Mr. Max Gergel, of Columbia Organic Chemicals Co., Columbia 99, S. C., by furnishing samples of decyl, dodecyl, and tetradecyl mercaptans. These products, described as being "95% pure," were purified by distillation at reduced pressure and were used as reference compounds.

captides may be stored for a reasonable period without the above changes occurring. The mercaptan may be regenerated from the lead mercaptide by warm hydrochloric acid treatment.

The water numbers (4) of the mercaptans were determined and compared with the hydroxy analogs.

EXPERIMENTAL

Preparation of the *n*-Alkyl Bromides.—The alkyl bromides with a carbon chain length from C_{10} to C_{18} were prepared in the manner described by Kamm and Marvel (11) for dodecyl bromide. To increase the yield of the hexadecyl bromide and the octadecyl bromide, the refluxing was stopped at the end of three hours and the layers separated. The acid layer was discarded and a fresh portion of 48% hydrobromic acid was added and the refluxing continued for an additional two hours. The yield of the various alkyl bromides obtained are given in Table I.

TABLE I.—THE ALKYL BROMIDES

Compound	B. P., 12–15 Mm.	Yield, %
$C_{10}H_{21}Br$	123–125	82
$C_{12}H_{25}Br$	148–150	70
$C_{14}H_{29}Br$	170–172	68
$C_{16}H_{33}Br$	193–195	63
$C_{18}H_{37}Br$	214–218	52

Preparation of the *n*-Alkyl Mercaptans.—In a liter flask, fitted with a reflux condenser, was placed 300 cc. of absolute ethanol. One-tenth mole of sodium (2.3 Gm.) was added and when solution was effected, hydrogen sulfide was passed through the reaction medium. When the mixture showed a gain in weight of 10 Gm., 0.1 mole of the appropriate alkyl bromide was added. The mixture was heated to reflux and the stream of hydrogen sulfide was continued through the reaction medium. It was then refluxed for six hours, cooled and filtered, and concentrated to one-third its original volume. The mercaptan separated on dilution with 150 cc. of ice water and was removed with the aid of a separatory funnel or by filtration (stearyl mercaptan). The mercaptans were purified by distillation at reduced pressure (Table II).

TABLE II.—*n*-ALKYL MERCAPTANS

Compound	B. P.,[a] 1–2 Mm.	% Yield[a]	% Yield[b]	2–4 Dinitrophenylthioether,[c] M. P., °
$C_{10}H_{21}SH$	108	61	66	89
$C_{12}H_{25}SH$	126	72	68	92
$C_{14}H_{29}SH$	149	68	71	93–94
$C_{16}H_{33}SH$	170[d]	70	64	95–96
$C_{18}H_{37}SH$	188[e]	66	58	97

[a] Sodium hydrosulfide method.
[b] S-Alkylisothiouronium Bromide method.
[c] Bost, Turner, & Norton, *J. Am. Chem. Soc.*, **54**, 1985 (1932).
[d] M. p. 17–19°.
[e] M. p. 24–26°.

The mercaptans were also prepared by reacting thiourea with the alkyl bromide in the manner described for the preparation of S-methyl-isothiourea sulfate (12). Absolute alcohol was used as the solvent and the period of reflux was lengthened to three hours. The melting points and yields are given in Table III.

TABLE III.—*n*-ALKYL-ISOTHIOURONIUM BROMIDES

n-Alkyl	M. P., °	Yield, %
$C_{10}H_{21}$	99	88
$C_{12}H_{25}$	103	86
$C_{14}H_{29}$	106	83
$C_{16}H_{33}$	110	84
$C_{18}H_{37}$	112	80

The mercaptan was liberated from the isothiouronium bromide by boiling with 5% sodium hydroxide in equimolar proportions. The product was extracted with ether, dried, and purified by distillation at reduced pressure (Table II). Approximately 10 to 18% of the dialkyl sulfide resulted from this method (Table IV).

TABLE IV.—THE DIALKYL SULFIDES

Empirical Formula	—Melting Point— Found	Received[a]	% Yield[b]	% Yield[c]
$(C_{10}H_{21})_2S$	24–25[d]	. . .	5	10
$(C_{12}H_{25})_2S$	33–34	33.5–34	4	14
$(C_{14}H_{29})_2S$	44–45	45.5–46	6	15
$(C_{16}H_{33})_2S$	51–52	53.5–54	6	12
$(C_{18}H_{37})_2S$	61–62	62.0–62.5	6	18

[a] Fore, D., and Bost, R. W., *J. Am. Chem. Soc.*, **59**, 2557 (1937).
[b] Sodium hydrosulfide method.
[c] S-Alkyl isothiouronium salt method.
[d] B. p. 178–180°, 1–2 mm. pressure.

Preparation of the Lead Mercaptide.—To 0.1 mole of the appropriate mercaptan dissolved in 250 cc. of hot absolute ethanol was added 50 cc. of a 5 normal alcoholic lead acetate solution. An immediate precipitation of the lead mercaptide resulted. The mixture was digested on a water bath for a half hour and then filtered while hot. The precipitated material was washed with hot water and then with absolute ethanol and dried. The dried mercaptides were extracted with boiling ether and then dried in a desiccator (Table V).

TABLE V.—THE LEAD MERCAPTIDES

$(C_nH_{(2n+1)}S)_2Pb$

Compound	M. P., °	Yield, %	—Pb, % Found	Calcd.
$(C_{10}H_{21}S)_2Pb$	98–100	88	37.3	37.4
$(C_{12}H_{25}S)_2Pb$	103–105	86	33.7	33.9
$(C_{14}H_{29}S)_2Pb$	104–105	86	31.0	31.1
$(C_{16}H_{33}S)_2Pb$	106–107	84	28.6	28.7
$(C_{18}H_{37}S)_2Pb$	109–111	82	26.4	26.6

The dried mercaptides were stored in an amber bottle and were fairly stable during the period of observation (two months). The mercaptans were regenerated as needed by treating the mercaptide with warm dilute hydrochloric acid and purified by distillation.

The water numbers of the mercaptans were determined in the manner described by Halpern and Zopf

(4) (Table VI). A correlation between the water numbers of the mercaptans and the hydroxy analogs with regard to carbon chain length was made (Fig. 1 and Fig. 2).

DISCUSSION

As a whole the mercaptan series caused rather little potentiation of the water absorption by petrolatum. The range in values obtained at the optimum concentration of the mercaptan was from 15 to 18. The hydroxy analogs ranged from 19 to 46 at their optimum concentrations. The water number at the optimum concentration continued to decrease slightly with the increase in the carbon chain length. The corresponding series of hydroxy derivatives, however, showed an increase in the water number with the increase of the carbon chain.

The optimum concentrations remained fairly constant in the range of 2 to 4%, and the decyl and tetradecyl mercaptans as well as the dodecyl and octadecyl mercaptans had the same optimum concentrations. The optimum concentrations of the corresponding hydroxy derivatives showed a decrease, with the increase in the carbon chain length, and ranged from 12.5 to 3%. The minimum, however, was at hexadecyl alcohol (4%) and there was a slight increase of the optimum with octadecyl alcohol (6%).

The poor emulsifying properties of the mercaptans may be explained, in part, by their rather limited tendency to associate. While it has not been conclusively established that hydrogen bonding does not occur with these *n*-alkyl mercaptans, it is generally accepted that the *n*-alkyl sulfhydryl compounds are nonassociated. The property of association or hydrogen bonding may well account, to some extent, for the superior emulsifying properties of the hydroxy analogs. The role of hydrogen bonding might be further established through infrared spectrum analysis. Further work is planned along these lines.

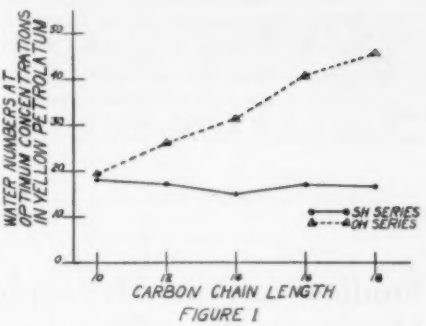

FIGURE 1

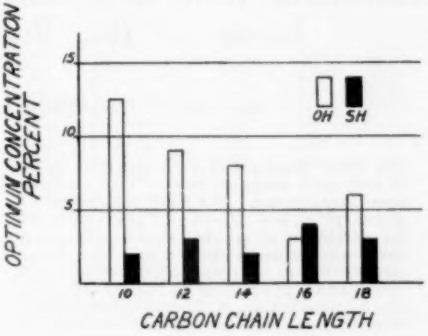

FIGURE 2

SUMMARY

1. The mercaptans with a carbon chain length from C_{10} to C_{18} were synthesized. The physical properties of these compounds and their intermediates were noted.

2. The water numbers of the mercaptans were determined and a correlation was made between the mercaptan series and their hydroxy-analogs.

3. The mercaptan series as a whole showed little ability to emulsify water in petrolatum. The range in water numbers for the mercaptan series was from 15 to 18 at the optimum concentration.

4. The optimum concentration of the mercaptans fluctuated slightly (from 2 to 4 per cent).

5. Since one contrasting difference between the series of mercaptans and the hydroxy analogs was the rather limited tendency of the sulfhydryl derivatives to form an associated complex, this might explain in part the rather superior properties of the fatty alcohols as emulsifiers.

TABLE VI.—THE WATER NUMBERS[a] OF THE *n*-ALKYL MERCAPTANS

Compound	Per Cent Concentration in Yellow Petrolatum[b]									
	1	2	3	4	5	6	7	8	9	10
$C_{10}H_{21}SH$	17	18	15.5	12.1	11.0	10.5	10.3	10.0	9.8	9.5
$C_{12}H_{25}SH$	14.2	16.1	17.3	14.3	13.1	11.1	9.8	9.6	9.4	9.1
$C_{14}H_{29}SH$	13.8	15.0	13.2	11.6	11.5	11.3	10.8	9.3	9.3	9.2
$C_{16}H_{33}SH$	13.2	15.1	16.3	17.1	14.2	13.1	12.0	10.3	9.6	9.2
$C_{18}H_{37}SH$	13.5	15.8	16.6	16.0	15.4	13.4	12.3	11.0	10.0	9.3

[a] Based on 100 Gm. of Mixture.
[b] Water number of the yellow petrolatum was 9.0.

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Studies on the Hydrophilic Properties of Possible Ointment Base Constituents. IV. The Fatty Acid Esters of the Polyhydroxy Alcohols*

By ALFRED HALPERN† and NICHOLAS SQUEGLIA‡

The water numbers of a homologous series of fatty acid esters of polyhydroxy alcohols were investigated. The fatty acid esters of glycol, propylene glycol and glycerol (with the exception of glyceryl-mono-oleate) did not cause emulsion formation at the lower concentrations. Glycol-monostearate and propylene-glycol-monostearate afforded slight emulsification at concentrations greater than 10 per cent. The fatty acid esters of sorbitan and mannitan are excellent emulsifying agents. These agents produced the greatest increase in the water number of petrolatum of all the compounds studied. This study was limited to the W/O emulsions.

AS PART of a study (1–3) of the hydrophilic properties of ointment base constituents in progress at these laboratories, a homologous series of fatty acid esters of the polyhydroxy alcohols was investigated. It was proposed (1) to develop a system of ointment base formulation that would enable the pharmacist to incorporate varying amounts of water or a solution of a medicament in accordance with the individual needs. A second phase of this study was to investigate the mechanisms of ointment formation and to determine the optimum concentration of the emulsifier that exerts the maximum effect as evidenced by the water number[1] of the fixed fat. The experimental agents used in this study have been investigated for emulsifying properties (5, 6). For the most part, these studies determined the effect of a fixed concentration of the emulsifying agent in the oil phase. No determination of the optimum concentration for emulsification activity was reported. Since the water number[1] of a mixture is an expression of the emulsification limits of the mixture, it was deemed important to determine these limits for a constant order of concentrations and note the optimum if it existed. A method of formulation would necessarily depend on these values and the flexibility of the use of these agents would be governed by these limits. It is emphasized that the preparations used are not to be construed as recommended ointment bases, nor is it proposed that the hydrated mixtures be interpreted as such. The mixtures are to be viewed as controlled experiments to determine the potentiation of the water numbers of the standard petrolatum due to the activity of the test compound.

EXPERIMENTAL

The water numbers of varying concentrations of ethylene glycol, propylene glycol, glycerin, sorbitol, a concentrated glucose syrup, and Simple Syrup, U. S. P. XIII, were determined in the manner described by Halpern and Zopf (1). All of these agents caused no potentiation of the water number of the petrolatum used. These agents all have a strong hydrophilic character and a significant absence of a hydrophobic group. It was expected that these agents would not evidence any emulsification properties because of the absence of the lipophile.

The fatty acid esters of the polyhydroxy alcohols used in this study were glycol-monostearate, pro-

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[1] The water number of a fixed fat was defined by Casparis and Meyer (4) to be the largest amount of water which 100 Gm. of an ointment base or fat will hold at normal temperature (20°).

pylene-glycol-monostearate, glyceryl-monolaurate, glyceryl-mono-oleate, glyceryl-monostearate, sorbitan-monolaurate,[2] sorbitan sesqui-oleate,[2] sorbitan-dioleate,[2] mannitan-oleate,[2] mannide-oleate,[2] and polyoxypropylene-mannitol-dioleate.[2]

The water numbers of these compounds were determined in the described manner (1) and the results were noted on Table I.

to litmus. The compounds prepared in this laboratory through the reaction between sodium stearate and epichlorhydrin and subsequent conversion to glycerol-1-stearate were neutral to litmus. The results reported in Table I were from neutral preparations.

The fatty acid sorbitan and mannitan esters greatly potentiated the water number of petrolatum.

TABLE I.—THE WATER NUMBERS OF THE FATTY ACID ESTERS OF THE POLYHYDROXY ALCOHOLS

Compound	Per Cent Concentration in Yellow Petrolatum[a]							
	1	2	3	4	5	7.5	10	12.5	15
Glycol monostearate[b]	No emulsion							14.1	14.2
Propylene glycol monostearate[b]	No emulsion						12.2	12.6	13
Glyceryl monolaurate[b]	No emulsion								
Glyceryl mono-oleate[b]	28	35	40	48	56	61	69	74	79
Glyceryl monostearate[b]	No W/O emulsion								
Sorbitan monolaurate	54	55	58	64	64	64	64	...	...
Sorbitan monopalmitate	64	69	76	84	92	128	150	180	241
Sorbitan monostearate	86	194	294	296	341	400	428	451	473
Sorbitan mono-oleate	360	440	605	740	900	1040	1180	1390	1470
Sorbitan sesqui-oleate	290	360	480	590	792	1010	1240	1340	1480
Sorbitan dioleate	491	482	601	716	800	981	1080	1110	1130
Mannitan oleate	295	361	400	481	580	651	1050	1200	1370
Mannide oleate	240	300	380	470	510	673	794	961	1121
Polyoxypropylene mannitol dioleate	Oil in water emulsion								

[a] Water number of petrolatum used was 10.1.
[b] Prepared in our laboratory. The *p*H of the 5 and 10% mixtures in water ranged from 6.8 to 7.2.

DISCUSSION

Glycol-monostearate and propylene-glycol-monostearate failed to produce W/O emulsions up to 10% concentration. The glycerol esters gave a rather poor potentiation of the water number when compared to the fatty acid esters of sorbitan and mannitan. This was rather surprising as the molecule had the usual requisites for an emulsifying agent, a hydrophilic group and a lipophilic group. Similar effects have been reported by Griffin (5).[3] No emulsion was obtained by him with glycol-mono-oleate and propylene-glycol-mono-oleate and glyceryl-monostearate (7). The trade literature suggests that small amounts of alkali be added to the aqueous phase for improved emulsification with some of these agents (8). This was done with a sample of 5% glycerol-monostearate in petrolatum. The addition of 0.1% alkali as sodium carbonate or sodium hydroxide to the aqueous phase resulted in a fairly good uptake of water by the mixture. The emulsion type was determined and was found to be O/W in contrast to the W/O type formed by the other esters *per se*. Since this study was confined to the mechanisms of the W/O emulsions, this effect was not further investigated in this study. It is of interest to note that some samples of commercially available glycol and glycerol esters from different sources and described as "self-emulsifiable" were found to be strongly alkaline in reaction

These compounds caused a water uptake up to 1200% (based on the 10% mixture). These agents appear to be superior to any of the agents tested. These compounds were unique in that the molecule contained both a hydroxyl group and an oxygen in an ether linkage. In addition to the groups contributing to the hydrophylic and lipophylic character of the molecule, there was also a cyclized nucleus resulting from anhydrization in their synthesis. The chemistry of these compounds is discussed in the trade publication describing their properties (9).

These agents apparently had no optimum concentration within the limits studied. The water number continued to increase with the increase in concentration. There appeared to be a leveling off of the rate of water number increase at about 10%.

It is particularly interesting to note the effect of the increase of hydrophilic groups on the molecule as evidenced by the water absorption of the mixture. It might be considered that the increase in the hydrophilic groups strengthens the bond between the hydrophile and the water at the same time weakening the attraction of the lipophile for the oil phase. The additional increase of the hydrophilic groups to the molecule so weakens the hydrophobe-oil bond that separation occurs. This factor may be demonstrated by the addition of polyoxyethylene groups to sorbitan mono-oleate. The resultant polyoxyethylene sorbitan mono-oleate gave no W/O emulsion in contrast to the excellent emulsifying properties of the sorbitan mono-oleate (7). Polyoxypropylene mannitol dioleate formed a fairly stable oil in water emulsion. The water number cannot be determined for an O/W emulsion.

The increased hydrophilic character of the fatty acid glycols in contrast to the fatty acid esters of the

[2] Furnished by the Atlas Powder Co., Wilmington 99, Del., through the courtesy of William C. Griffin of the Central Research Laboratory. These products are commercially available under the registered trade mark of "Arlacel," "Span," and "Tween."
[3] The emulsifying efficiency of glycerol-mono-oleate was reported (5) to be significantly greater than the value we found (Table I).

polyhydric alcohols might well explain the difference in emulsifying ability exhibited by these agents. In the W/O emulsions a determinant of emulsion efficiency might be the strength of the lipophile-oil bond. With the increased hydrophilic nature of the fatty acid glycols, the lipophile-oil relationship was weakened so that no W/O emulsion formed.

The saturated hydrated mixtures had rather poor pharmaceutical properties. There was a great loss of water, both by separation and evaporation, on storage at 50° for eight hours. The spreading of the hydrated mixtures was not satisfactory. While it is recognized that these mixtures were not intended to be used as ointment bases, their superior hydrophilic properties made them excellent starting materials for the formulation of bases that could be adjusted to the individual needs.

Preliminary experiments indicated that the separation of water from these mixtures could be minimized by reducing the hydration to 50–75% of saturation. The addition of small amounts of white wax improved the spreading qualities of the mixtures. The emulsifying agents were stable to dilute acid and dilute alkali at room temperature. They were compatible with such heavy metal salts as silver nitrate and mercuric chloride. The *p*H of the commercially available preparations ranged from 5.8 to 7.4. These agents apparently are nonirritating to the skin (10). When applied to the inner surface of the forearm for fourteen days no irritation was experienced. Work now in progress on the adjustment of these mixtures into suitable ointment bases will be reported at a later date.

SUMMARY

1. The water numbers of a homologous series of fatty acid esters of polyhydroxy alcohols was investigated.

2. The fatty acid esters of glycol, propylene glycol, and glycerol caused no emulsion formation at the lower concentrations, with the exception of glyceryl-mono-oleate. Glycol-monostearate and propylene-glycol-monostearate afforded slight emulsification at concentrations greater than 10 per cent.

3. The fatty acid esters of sorbitan and mannitan are excellent emulsifying agents. These agents produced the greatest increase in the water number of petrolatum of all the compounds studied.

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Color Reactions for Copalquin Bark Extract*

By JACOB A. SCHULDINER†,‡

The source, preparation, and use of copalquin bark extract as an adulterant and substitute for raw and smoking opiums is discussed. A series of qualitative tests are used to distinguish it from crude or smoking opiums and for the detection of admixtures.

MANY adulterants have been found in raw and smoking opiums. Recently a new one was alleged to have come from Mexico. This product, so-called "gum copalquin," has the odor, color, consistency and general appearance of opium. Its principal use has been for the adulteration of smoking opium. It has also been sold both as raw and as smoking opium without the admixture of genuine opium.

It has become necessary to distinguish this material and its mixtures with opium products from the pure narcotics. In order to accomplish this, the source material was identified, the method involved in its production was discovered, and a series of chemical tests for its identification and differentiation from opium were worked out.

SOURCE OF MATERIAL AND PREPARATION OF THE EXTRACT

While on a trip into Mexico, the author obtained authentic source materials from which gum copalquin is made. He was also able to confirm the identification of the substance as bark from the copalquin tree, *Coutarea pterosperma* (1, 2).

An authentic sample of the bark of *Coutarea pterosperma* was coarsely ground. The powder was placed in a large fritted glass funnel and extracted with cold distilled water. To aid the flow of extract a little vacuum was applied occasionally.

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† The author gratefully acknowledges the assistance of a number of persons whose identity must remain anonymous.
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The extraction was continued almost to completion, as indicated by the light color of the final extract.

After considerable experimentation it was discovered that when the odorless extract was treated with small portions of powdered hydrated lime and boiled to the proper consistency, a product was obtained which had the characteristic odor and appearance of smoking opium. The alkali and heat caused the formation of free volatile substances which have characteristic amine-like odors. These substances are probably formed either by hydrolysis and decomposition of proteins or by their release from amine-like salts present in the bark extract.

From the identification of the source of this material and its method of manufacture, it has been concluded that it would be more accurately described as copalquin bark extract.

SPECTROGRAPHIC ANALYSIS

Spectrographic analysis of copalquin extract ash indicates that the pure extract could probably be distinguished from Mexican raw or smoking opiums. However, the differences are not so specific that this purpose could be accomplished with mixtures of copalquin bark extract with smoking opium, raw opium, or opium residues.

PROCEDURE IN TESTING FOR ADULTERATION

A series of color tests were made with various reagents. These tests were on a group of fifteen authentic raw opiums from a wide variety of known sources; on several samples of gum copalquin; and on seizures of alleged Mexican smoking opiums, two of which were suspected to contain copalquin bark extract.

The reagents used in the tests were:

(1) Concentrated NH_4OH
(2) Sodium hydroxide 1.0% in H_2O
(3) $Ca(OH)_2$, a saturated H_2O solution
(4) Concentrated H_2SO_4
(5) Concentrated HCl
(6) Phosphoric acid 85%
(7) Ferric ammonium sulfate, dilute solution in H_2O
(8) Lafon's reagent: 1.0 Gm. of ammonium selenite in 20 ml. of concentrated H_2SO_4.
(9) Iodine solution in petroleum ether. A few crystals of iodine dissolved in 50 ml. of extraction-grade petroleum ether.

These tests with the noted exceptions (ferric ammonium sulfate and iodine in petroleum ether) were made by adding the reagent directly to a small portion of the solid material in a test tube.

Gum copalquin is readily soluble in cold alkalies with the exception of 50% sodium hydroxide. The solution in alkalies is a clear, deep brownish yellow color. Opiums are usually much less soluble and the resulting color is lighter and often a muddy suspension is present.

Concentrated sulfuric acid gives a pink to deep red color with gum copalquin. When this is carefully diluted with water, the dilution is pink. Opiums give yellow to brown colors with concentrated sulfuric acid.

Concentrated hydrochloric acid gives pink to deep magenta shades with copalquin extract. With opiums, deep yellow colors are produced.

Phosphoric acid 85% gives characteristic pink to magenta colors with copalquin bark extract. With opiums, the colors are straw to deep yellow.

Ferric ammonium sulfate gives distinctive greenish brown precipitates with copalquin extract dissolved in distilled water. With opiums, the color is red-brown.

Lafon's reagent gives a red-brown color with copalquin extract and greenish brown with opiums.

Raw opiums give an orange color with iodine dissolved in petroleum ether.

The samples are dissolved in petroleum ether and filtered. The iodine reagent is added to the filtrate. Old, oxidized samples sometimes fail to give this reaction. If the hard outer portion is discarded, the unoxidized inner portion will usually respond to the test. With copalquin extract and smoking opiums, both of which are water extracts, the original purple color of the iodine reagent remains unchanged.

Mixtures of copalquin bark extract and opium are detected by the following criteria:

(1) Morphine content.
(2) Pink and red colors with H_2SO_4, HCl, and H_3PO_4, indicate the presence of copalquin bark extract.
(3) Increased solubility in alkaline reagents and deep yellow color often is a sign of copalquin extract adulteration.
(4) Mixed color reactions when compared with the color reactions of authentic copalquin extract and opium products are indicative of adulteration.
(5) The absence of morphine coupled with an orange color with the iodine in petroleum ether would indicate a mixture containing opium residues.
(6) Raw opium, in mixtures with copalquin extract, is indicated by the orange color with petroleum ether-iodine reagent, if morphine is also found present.
(7) Smoking opium, in mixtures with copalquin extract, is indicated by the morphine content coupled with a negative (purple) color with petroleum ether iodine solution.

CONCLUSIONS

Gum copalquin has been identified by means of authentic materials and chemically as an alkalized water extract of the bark of *Coutarea pterosperma*. The method of preparation of the extract has been determined. A series of tests have been evolved, which, combined with a quantitative determination of the morphine content, can be used to prove the adulteration of various opium products with copalquin bark extract.

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Note

An Inexpensive Pen for Kymographic Recording*

By THOMAS J. HALEY

THERE are times when those who work with isolated smooth muscle preparations desire, for recording muscular contractions, some method which does not employ the smoked kymograph drum. Several expensive ink-writing instruments are available but their continuous use requires more than the usual amount of care to insure a continuous flow of ink to the recording surface. A simple glass tip ink writer has also been used, but this is subject to breakage.

For the last two years this author and several of his colleagues have employed the commercially available "Beta" fountain pen[1] for recording both smooth muscle contractions and respiration in anesthetized animals. The pen is attached to the smooth muscle lever by a small drop of solder or plastic cement. It may also be attached by filing small holes in its supporting arm and inserting the muscle lever therein. If necessary a reduction in the weight of the simple type lever arm may be accomplished by using a hollow aluminum tube such as is used in making model airplanes. Under these circumstances the lever holder is reamed out to accommodate the larger diameter of the tube.

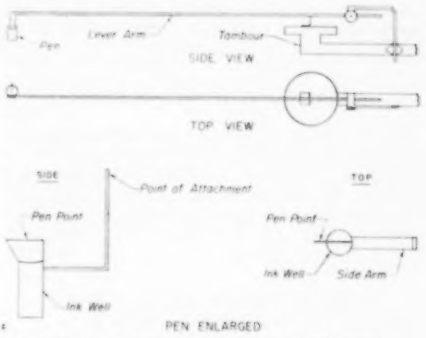

Fig. 1.—Pen and method for attachment.

This latter method is usually used when the pen is attached to the lever arm of the tambour used to record respiration. See Fig. 1 for a diagram of pen and method of attachment.

It is unfortunate that the weight of this simple pen prevents its use in recording blood pressure with a mercury manometer. Any attempt to use it for that purpose usually results in the damping of the pressure wave and no record. However, the pen might be useful with a Harvard membrane manometer.

* Received October 18, 1948, from Medical School, University of California at Los Angeles.

[1] American Recording Chart Co., 3113 East 11th Street, Los Angeles 23, Calif.

Book Reviews

The Basis of Chemotherapy. By THOMAS S. WORK and ELIZABETH WORK. Interscience Publishers, New York, 1948. xx + 435 pp. 14 x 22 cm. 42 illus. 38 tables. Price $6.50.

Beginning with his interest in the distribution of dyestuffs in the animal body in 1885, and culminating with the discovery of "606" in 1911, the works of Ehrlich form the true foundation for chemotherapy. After the discovery of arsphenamine there followed an intense search for chemotherapeutic agents capable of attacking bacteria as well as protozoa. Indifferent success in this area almost caused the field to be abandoned until the spectacular sulfonamides arrived on the scene. This rebirth brought with it an intensified study of the mechanism of drug action resulting in the competitive inhibition concept, so applicable to fields like the antihistaminics as well as infectious diseases.

Because of the intensified interest in chemotherapy during the last fifteen years or so, it is fitting that someone should review the field at this time. This has been accomplished by the Doctors Work who have succeeded in covering the field with a fair measure of thoroughness without the narrow-

ness of simply recording factual data. Consistently throughout the book is found a stimulating viewpoint.

After a very excellent introductory chapter on the history of chemotherapy, the author discusses cell metabolism, the biochemistry of essential metabolites and enzyme inhibition. These chapters form an excellent prologue to those following on drug antagonism, drug resistance, and the relation of structure to the activity of drugs.

This well-developed treatise will widen the perspectives of all workers interested in the hybrid field of chemotherapy.

Pharmacology. 3rd edition. By J. H. GADDUM. New York, 1948. Oxford University Press. xvi + 504 pp. 13 x 22 cm. Price $8.

The third edition of this text follows the same pattern as previous editions. Characteristic of the main additions and changes are folic acid, action of drugs on endocrine glands, drugs acting against acetylcholine, epinephrine, and histamine, anticoagulants, BAL, insecticides, detergents, sulfonamides, streptomycin, and penicillin.

Due to brevity, this text has limitations in the teaching of practical pharmacology to physicians and pharmacists. To those interested in experimental pharmacology, it is a very useful book, however. Of particular advantage is the modernized section on the use of statistics in bioassay.

The Biological Standardization of the Vitamins. 2nd edition. By KATHERINE H. COWARD. The Williams and Wilkins Co., Baltimore, 1947. vii + 224 pp. 13.5 x 23.5 cm. Price $5.

The Biological Standardization of the Vitamins is divided into two parts. Part I is a practical portion of the book. After two chapters dealing with general principles, the bioassays of the individual vitamins are discussed. This portion of the book terminates with a chapter on the interdependence of the vitamins.

Part II is more theoretical in nature and is concerned with a statistical treatment. In separate chapters the standard deviation, factors influencing the accuracy of vitamin determinations, the limits of error, and improvement of bioassays are discussed here. Considerable revision, based on research by Irwin, Bliss, Fieller and others, is in evidence.

Specialists in the field will find much of interest in this book.

Textbook of Pharmacognosy. 6th edition. By HEBER W. YOUNGKEN. The Blakiston Company. Philadelphia, 1948. 1063 pp. 15 x 23 cm. Price $8.50.

Perhaps no other division of the medical sciences has advanced more in the last decade than that dealing with the drugs of vegetable and animal origin. The introduction of antibiotics and vitamins, the "rediscovery" of a few drugs, such as pareira, cocculus, and podophyllum, and the purification of more of the plant constituents, such as glycosides and alkaloids, are reasons for revisions of works on pharmacognosy. A revision is also convenient, if not necessary, to keep pace with the Pharmacopœia.

The cutting off of supplies of cinchona from Java by World War II stimulated search for and the finding of many sources in Central and South America. It also encouraged experimental growing of cinchona in the Western Hemisphere.

The discovery and history of cinchona in the seventh century, while dealt with briefly by this author, adds much interest to the study of such an important drug. Cinchona and quinine production undoubtedly involve the application of more sciences than any other crude drug. Dr. Youngken has brought these topics up to date in a very usable form.

Allergy and pollen have been given the attention they deserve in a textbook of pharmacognosy.

It is thought that the discussions on vitamins should be much more complete than those found in this text.

Digitalis lanata is past the experimental stage and it deserves the attention given it in this book. This unofficial species of Digitalis, with actions quite similar to those of the official species, and with two to five times its potency, enables the physician to make a wider selection of heart tonics. The importance of Digitalis lanata is shown by the fact that two of its glycosides were introduced into U. S. P. XIII.

The increased importance of endocrine glands and their hormones justifies the enlargement of this section of the book, adding valuable illustrations and lengthy descriptions of both the whole and powdered glands. While the student will study the pharmacology of the endocrines later in his curriculum, their inclusion in the course in pharmacognosy enables the student to study them as a distinct group of drugs and compare them with other animal drugs as to origin, constituents, and other details.

Many authors of textbooks fail to realize the value of the history of their subject as a stimulant to the student's interest. Professor Youngken has a very good but much condensed history of pharmacognosy which might well be enlarged to include such topics as the very earliest writers on American drugs including Monardes, Martin de la Cruz, and David Schoepf.

It is suggested that Chapter III be divided into one chapter on antibiotics and one on bacterial biological products, and that each have an introduction to bring out the point that the inclusion in medicine of these groups was a great stride in the advance of medicine. The introduction to the second of these chapters might be greatly enhanced by a discussion of Louis Pasteur, who was the Father of Immunology.

The author has treated the antibiotics in a very thorough way. It would be unwise to attempt to list all of the antibiotics here or to give a complete discourse on any one, but the author has included concise monographs on penicillin, streptomycin, tyrothricin, chlorophyll, and bacitracin.—CHALMERS ZUFALL.

Lectures on the Inorganic Nutrition of Plants. Second Printing. By D. R. HOAGLAND. The Chronica Botanica Co., New York City. Stechert-Hafner, Inc., 1948. 226 pp. 14.5 x 23 cm. Price $4.50.

The subject of this volume is Dennis R. Hoagland's plant nutrition lectures which were presented

at the recent Prather Lectureship series at Harvard University. The material is abundant in data of interest to students and teachers alike in the important functions and mechanisms by which inorganic elements affect plant life. Seven lectures are edited in splendid style covering first a survey of plant nutrition problems, the role of the nutrients, potassium and calcium, and the micronutrients, zinc, manganese, cobalt, boron, copper, iron, and molybdenum, in plant functions. This is followed by lectures on special phases of plant physiology and plant chemistry. Included in the latter series are the absorption and accumulation of salts by cells, movement of inorganic solutes in plants, and various plant nutrition factors involved in water and sand culture media. Of great significance to the pharmacist is lecture Number Six. Herein is a discussion of some biochemical problems associated with salt absorption, organic acids, nitrogen, protein and ion accumulation since these factors influence chemical processes throught to be important in plant biosynthesis. The author might have speculated further to the advantage of the plant chemist. The review of the Krebs cycle and the allusion to the importance of ammonia in plant amide synthesis are helpful.

Only very general observations are offered relative to the field of radio active isotopes as tools in plant nutrition problems. In fact the reader finds himself wishing for more on this subject, especially data dealing with the methods. Nevertheless many useful references are included. In fact each lecture is well referenced at its conclusion.

The series is accompanied by twenty-eight plate-photographs showing experimental plant growth observations covering certain aspects of the text. Most of these appear to be of material which is under experimentation in the Division of Plant Nutrition Laboratories, University of California. The pharmacognosist or plant chemist will gain much from each of Hoagland's lectures and, more important still, he will find himself continuously in a speculative mood during his reading.—HEBER W. YOUNGKEN, JR.

International Rules of Botanical Nomenclature. By W. H. CAMP, H. W. RICKETT, and C. A. WEATHERBY. Waltham, Massachusetts: The Chronica Botanica Company. New York City: Stechert-Hafner, Incorporated, 1948. 120 pp. 17 x 26 cm. Price $3.50.

Plant taxonomists and allied botanists have long had need of an up-to-date compendium of the International Rules of Botanical Nomenclature. In this text the authors have assembled those major articles of the "Rules" initiated during the Botanical Congress at Amsterdam (1935) together with those recommendations for further changes in nomenclature which have since been proposed by recent International Botanical Congresses. The text is not intended to be an official printing of the format of the third (1935) edition of the "Rules." Nevertheless it follows the official form closely. Even the casual taxonomist will find stimulation in the many proposed recommendations. The index of conserved and rejected generic names in Appendix III is a decided feature.—H. W. YOUNGKEN, JR.

A Catalogue of Insecticides and Fungicides. Volume II. Chemical Fungicides and Plant Insecticides. By DONALD E. H. FREAR. The Chronica Botanica Co., Waltham, Mass., 1948. xii + 153 pp. 19.5 x 27 cm. Price $5.50.

For review of Volume I see THIS JOURNAL, 37, 214 (1948).

Dr. Frear set up a project at Pennsylvania State College to catalogue all substances which have insecticidal and fungicidal properties as recorded in the literature. Like Volume I, Volume II gives the chemical names, synonyms, and complete formulas as well as the results of tests on insects and fungi for chemical fungicides and plant insecticides. References and author indexes, patent lists, and a complete index of compounds listed in both volumes are present. Keys to the literature of this type are useful to both chemists and entomologists.

Elsevier's Encyclopedia of Organic Chemistry. Edited by F. RADT. Series III, Carboisocyclic Compounds, Volume 12B, Part I, Naphthalene. Elsevier Publishing Company, New York, 1948. xxx + 344 pp. 16 x 25.5 cm. Price: Subscription price $24; series price $28; single price $32.

For some time many chemists have wished for an English "Beilstein" which would augment, and, to a certain extent replace, the original. This is one of the early volumes of an encyclopedia to be produced to answer such a need. The compilers started with a volume other than "I" in order to make available literature not so readily accessible in "Beilstein" at the present. This volume, for example, covers the general literature up through 1944 and the literature concerning the structure of compounds through 1948.

The editors have kept in mind the fact that the organic chemist no longer works in isolation, and have introduced data of interest to the physicist and those working in the many fields of biology.

The arrangement of compounds is simple and the system is set up so that compounds most closely related are placed together. In connection with each compound, its additive derivatives are described—the only exception being where the derivatives belong to a very important heterocyclic ring system.

The generous use of tables makes it easy for the reader to get information quickly. For example, on Pages 12–15 is a very complete tabulation of the solubility of naphthalene in a variety of solvents at different temperatures. The table is usefully arranged and well documented.

Wherever possible information is given as to the physical properties of a compound, its chemical preparation, its stability, analytical properties, physiological action, and the preparation of principal derivatives.

Each volume carries a complete subject and formula index.

The typography is excellent and the type size is conducive to easy reading. Like "Beilstein," the value of this work lies in its completeness. If the other volumes maintain this high standard and become available soon, research libraries and laboratories will have a gold mine of chemical information easily accessible.

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